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14. ABSTRACT

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), is a major human pathogen causing significant human suffering worldwide. There is an urgent unmet need for new therapeutic agents and an increased understanding of the disease process. We propose to develop anti-tubercular agents that work in new ways by targeting protein secretion, a key virulence mechanism, and to understand how these compounds kill bacteria.

We previously identified several novel anti-tubercular series using a high throughput screen directed at protein secretion in live cells. We selected a set of representative compounds for each of these five series and commenced testing compounds for activity against multiple strains of *M. tuberculosis* which under-express LepB. We generated recombinant strains of *M. tuberculosis* to monitor protein secretion using a reporter protein (alkaline phosphatase). We screened our over-expression library against key molecules to identify potential resistance mechanisms and determined activity of compounds on agar plates in preparation for isolating resistant mutants.

We designed and synthesized a new set of analogs for the hydrazine series and tested these for both anti-tubercular activity and cytotoxicity. Several potent molecules were identified but these are also cytotoxic. We designed a second set of molecules aimed at reducing cytotoxicity while retaining potency. We designed new analogs for two additional series and prepared synthetic schemes.

We initiated studies to understand the essentiality and function of LepB in *M. tuberculosis.* We constructed new plasmids to generate controllable knockdown strains of LepB by CRISPRi in *M. tuberculosis.* We generated recombinant strains of *M. tuberculosis* with mutant alleles of LepB, which demonstrate that 1 of 6 cysteine residues in the protein is essential. In addition, we demonstrated that accumulation of ethidium bromide is reduced in our current LepB under-expressor strains, and that this is not linked to changes in efflux.

15. SUBJECT TERMS

Tuberculosis, anti-bacterial, anti-tubercular, drug discovery, infectious disease, protein secretion

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1. INTRODUCTION:

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), is a major human pathogen causing significant human suffering worldwide, with 1.8 million deaths and 10.4 million new cases in 2016. TB is of significant concern to the military, since TB is contagious and units are regularly deployed to areas where TB is endemic. There is an urgent unmet need for new therapeutic agents and an increased understanding of the disease process. We propose to develop anti-tubercular agents that work in new ways by targeting protein secretion, a key virulence mechanism, and to understand how these compounds kill bacteria. Protein secretion is the mechanism by which bacteria excrete or export proteins from inside the cell to the outside; it is important for both the survival of M. tuberculosis and for its ability to cause disease. Disrupting this mechanism could lead to new drugs to treat TB in people.

2. KEYWORDS:

Tuberculosis, anti-bacterial, anti-tubercular, drug discovery, infectious disease, protein secretion

3. ACCOMPLISHMENTS:

What were the major goals of the project?

	Timeline	**Complete**
Specific Aim 1. Develop a pharmacophore model for LepB inhibitors and generate lead compounds.	Months	
Identification of targets for each series.	1-17	50%
Identification of the pharmacophore for LepB	1-17	75%
Evaluation of series potential for lead generation	12-29	25%
Lead generation	25-36	25%
Milestone(s):		
Series selection for lead generation	29	
Lead selection	36	Prish
Specific Aim 2. Determine the mechanism of kill induced by LepB inhibitors.		
Determine if cell death is induced by accumulation of proteins in the membrane	1-17	60%
Determine if LepB inhibition changes cell wall structure and/or permeability	1-17	75%
Determine why cell death is more rapid under non-replicating conditions	17-29	25%
Determine if cell death kinetics vary under other conditions	17-29	10%
Identify key perturbations in the secretome	25-36	10%
Milestone(s):		
Determination of mode of action/mechanism of kill	29	
Determination of downstream perturbations of kill	36	Parish
Specific Aim 3. Determine the role of LepBMtb under conditions relevant to infection and identify the key residues required for proper LepBMtb function.		
Determine the role of key domains in LepB function	1-17	30%
Determine the role of cysteine resides in LepB function	1-17	50%
Determine the species –specificity for LepB	17-29	
Determine if LepB expression is conditionally essential	17-29	
Identify secretome changes in response to LepB knockdown	25-36	10%
Milestone(s):		

Identification of structural features of LepB	29	
Validation of LepB as a drug target under multiple conditions	36	

What was accomplished under these goals?

Our overall goal is to develop new anti-tubercular drugs that work by targeting protein secretion, a key virulence mechanism, and to determine the mechanism by which these agents kill *Mycobacterium tuberculosis*.

Our major objectives for the first year were to:

Objectives	**Timeline (months)**
Identification of targets for each series.	1-17
Identification of the pharmacophore for LepB	1-17
Evaluation of series potential for lead generation	12-29
Determine if cell death is induced by accumulation of proteins in the membrane	1-17
Determine if LepB inhibition changes cell wall structure and/or permeability	1-17
Determine the role of key domains in LepB function	1-17
Determine the role of cysteine resides in LepB function	1-17

We have made progress in each area, although the Covid-19 pandemic did have an impact on our work (see section on changes).

Specific Aim 1. Develop a pharmacophore model for LepB inhibitors and generate lead compounds. Our aim is to both determine the protein target(s) for each series, and to explore the pharmacophore for activity for compound series which target LepB. We will conduct studies to assess whether the target is LepB itself, another component of the secretory pathway, or a secreted protein. We will conduct structure-activity relationship (SAR) and structure-property relationship (SPR) for our novel series in order to identify the pharmacophore, to determine if the series are suitable for drug development, and to progress our hit compounds towards lead optimization. This will enable us to prioritize series for progression based on target tractability, overlap and druglike properties. We will select one series for progression into lead generation.

1.1 Identification of targets for each series

We are using three complementary approaches to identify series targeting secretion (i) use of a secretion assay using membrane fractions, (ii) use of a reporter protein to measure secretion in whole cells and (iii) monitoring changes in the secretome. Over the last year we focused on (i) and (ii). For (ii) we are developing a secretion reporter assay for *M. tuberculosis.* We have generated an alkaline phosphatase reporter fused to a *M. tuberculosis* signal peptide. We have transformed the plasmid into *M. tuberculosis.* We have also identified an alternative system using a secreted beta-lactamase and are in the process of acquiring a beta-lactamase deficient strain and the appropriate plasmids. For (iii) we have optimized our method to isolate culture filtrates from virulent *M. tuberculosis* using protein-free medium.

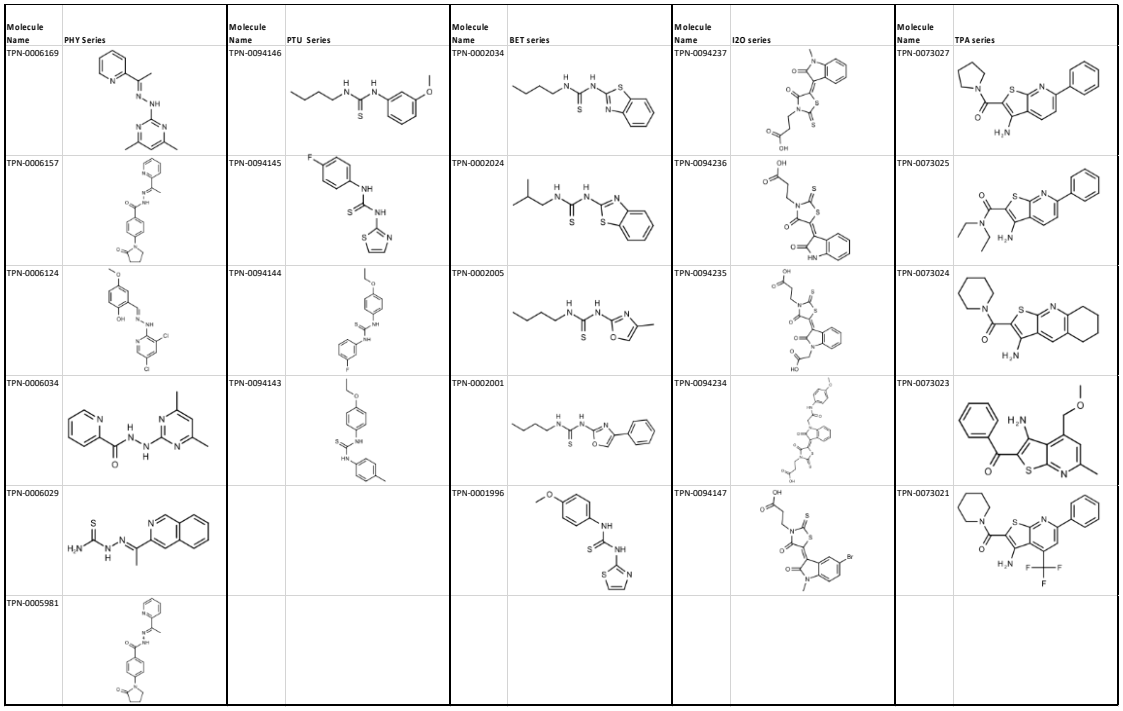
We are pursuing target identification using a number of approaches already used with some success in our group. Our main objectives have focused on (i) characterization of resistant mutants and (ii) screening of an over-expression library. For (i) we determined solid MICs for active compounds, so we can proceed with isolating resistant mutants for target confirmation (see section 1.4 for results from PHY series). For (ii) we have screened the entire over-expression library of 1700 clones for resistance to three compounds (1 each from the PTU, I2O and BET series) in liquid medium. The library was pooled and exposed to 100 µM of each compound over 21 days; viable bacteria were isolated on solid medium. Colonies were pooled and will be checked for resistance before sequencing.

Series	Cpd ID	Solid MIC (microM)
BET	TPN-0001996	100
BET	TPN-0002005	6.3
BET	TPN-0002034	50
I20	TPN-0094235	100
I20	TPN-0094236	3.1
PTU	TPN-0094145	25
PTU	TPN-0094143	25
PTU	TPN-0094146	50

Compounds from each series were tested for activity against wild-type *M. tuberculosis.* The minimum inhibitory concentration (MIC) was determined on solid medium and defined as reducing growth by >99% after 21 days.

1.2 Identification of the pharmacophore for LepB

We selected a set of key compounds for each of our priority series for profiling against the LepB under-expressor strains already in hand. These were selected to run through all assays based on activity and availability, as well as structural diversity.



Representative compounds from each series

We obtained supplies of molecules and are testing them against all of the LepB under-expressor strains in order to confirm on-target activity. We have generated a partial data set (see below). The strains in use are listed – each of which expresses LepB from a heterogenous promoter with lower expression levels than the wild-type strain. Once we have completed the dataset we will prioritize the series for evaluation.

M. tuberculosis strains	
H37Rv	wild-type ATCC25618
SPAM13C	chromosomal *lepB* Δ; integrated [PlepB-*lepB*, L5 int, Sm]; pCHERRY10 [mCherry, Hyg]
SPAM15C	chromosomal *lepB* Δ; integrated [Pgln *E*-*lepB*, L5 int, Sm]; pCHERRY10 [mCherry, Hyg]
SPAM17C	chromosomal *lepB* Δ; integrated [PRv2466c-LepB, L5 int, Sm]; pCHERRY10 [mCherry, Hyg]
SPAM18C	chromosomal *lepB* Δ; integrated [PRv2745c-*lepB*, L5 int, Sm]; pCHERRY10 [mCherry, Hyg]
SPAM19C	chromosomal *lepB* Δ; integrated [PRv2930-*lepB*, L5 int, Sm]; pCHERRY10 [mCherry, Hyg]
SPAM20C	chromosomal *lepB* Δ; integrated [PsenX3-*lepB*, L5 int, Sm]; pCHERRY10 [mCherry, Hyg]
SPAM22C	chromosomal *lepB* Δ; integrated [PtrpE-*lepB*, L5 int, Sm]; pCHERRY10 [mCherry, Hyg]
SPAM23C	chromosomal *lepB* Δ; integrated [PtrpD-*lepB*, L5 int, Sm]; pCHERRY10 [mCherry, Hyg]

Molecule Name	MIC (microM)			
	SPAM15	SPAM18	SPAM20	SPAM23
TPN-0094237		3.3		5.4
TPN-0094236		14		20
TPN-0094235		12		10
TPN-0094234		5.7		7.9
TPN-0094147	2.9	5.4	3.4	6.3
TPN-0094146		22		13
TPN-0094145		8.3		10
TPN-0094144		43		11
TPN-0094143	8.1	17	4.1	3.7
TPN-0006034	12	2.6	4.9	20
TPN-0002034	8.1	4	7.6	68
TPN-0002005	3.4	1.8	5.2	4
TPN-0001996	13	4.3	11	18

Compounds from each series were tested for activity against recombinant *M. tuberculosis.* The minimum inhibitory concentration (MIC) was determined in liquid medium and defined as reducing growth by >90% after 5 days.

Recent developments in CRISPRi technology have been applied to mycobacteria and a robust system for controllable and tunable gene expression is now available for *M. tuberculosis*. We have initiated additional studies to generate LepB knock down strains using this technology. Since gene expression can be controlled in the same strain simply by modifying the concentration of the inducer anhydrotetracycline (ATc), these strains will be more extremely useful for our studies. For example, we will be able to run checkerboard studies with compounds and ATc to determine precisely the effect of under expression of LepB on compound activity. We have constructed three CRISPRi plasmids incorporating different PAM sequences to fine tune the level of knockdown and transformed these into *M. tuberculosis*. We are characterizing these strains to confirm the level of under expression of LepB. Once we have confirmed these strains we will use these in subsequent experiments in place of the constitutively expressing LepB under-expressor strains to determine compound activity and conditional essentiality.

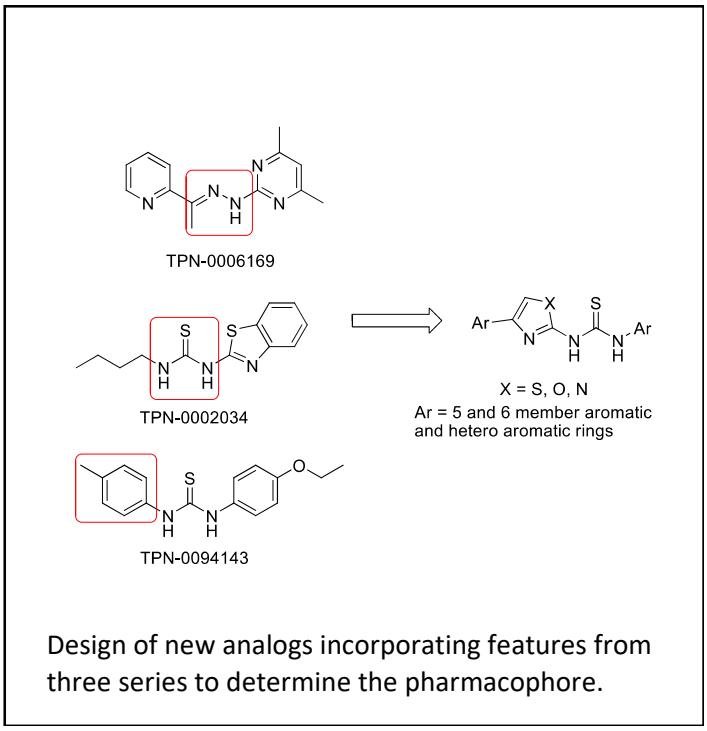
1.2 Evaluation of series potential for lead generation

We selected a set of key compounds for each of our priority series for profiling against the LepB under-expressor strains already in hand (see above). We have generated MICs and cytotoxicity data for new molecules.

1.4 Lead generation

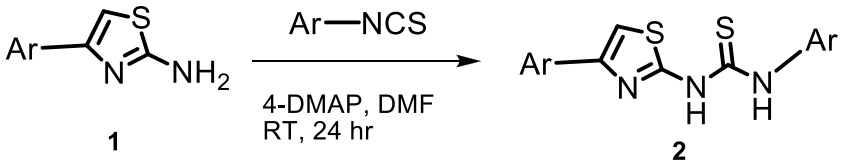
Our aim is to conduct thorough structure-activity and structure-property relationship studies for one series. Novel analogs will be tested in a variety of assays. We will evaluate hit series and progress through lead generation using a range of assays. We will conduct SPR studies and optimize the pharmaceutical properties required for *in vivo* exposure to facilitate an assessment of the therapeutic potential of the compound series against tuberculosis.

We evaluated each of the five series for chemical tractability and potential for progression and prioritized the phenyl hydrazine series for optimization. The key issue is to remove the hydrazine moiety while retaining biological potency. We designed a number of analogs which incorporate bioisosteres of the hydrazine. To address cytotoxicity, we planned to replace the hydrazine with a cyclized pyrrole ring. We incorporated SAR information from the BET and PTU with the PHY series, to design novel analogs for a thiourea series. These designs were aimed at reducing cytotoxicity, while retaining potency.

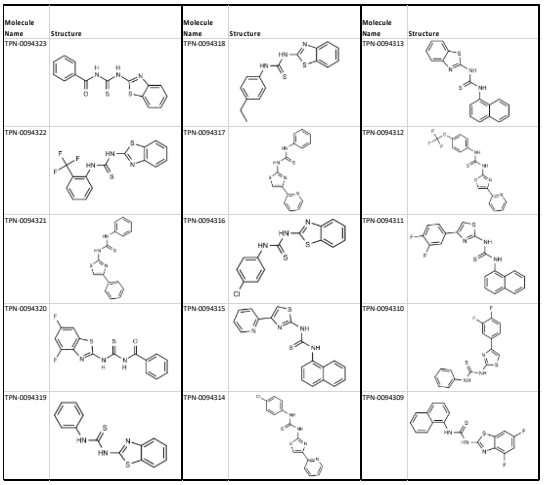


Design of new analogs incorporating features from three series to determine the pharmacophore.

A simple synthetic scheme (below) was used where reactions between the isothiocyanate and amine in the presence of a tertiary amine as a base in a polar solvent such a *N*,*N*-dimethylformamide (DMF) was used. Using this experimental procedure, we were able to synthesize and purify 15 compounds (~100 mg each). Compounds were verified by LC/MS for purity and mass and will be subjected to NMR characterization.



We tested new analogs for activity against *M. tuberculosis* and cytotoxicity against human HepG2 cells. From our first set, we obtained molecules with potency against the wild-type strain, but they still were cytotoxic. Two molecules lacked cytotoxicity, but these were also inactive against *M. tuberculosis.*

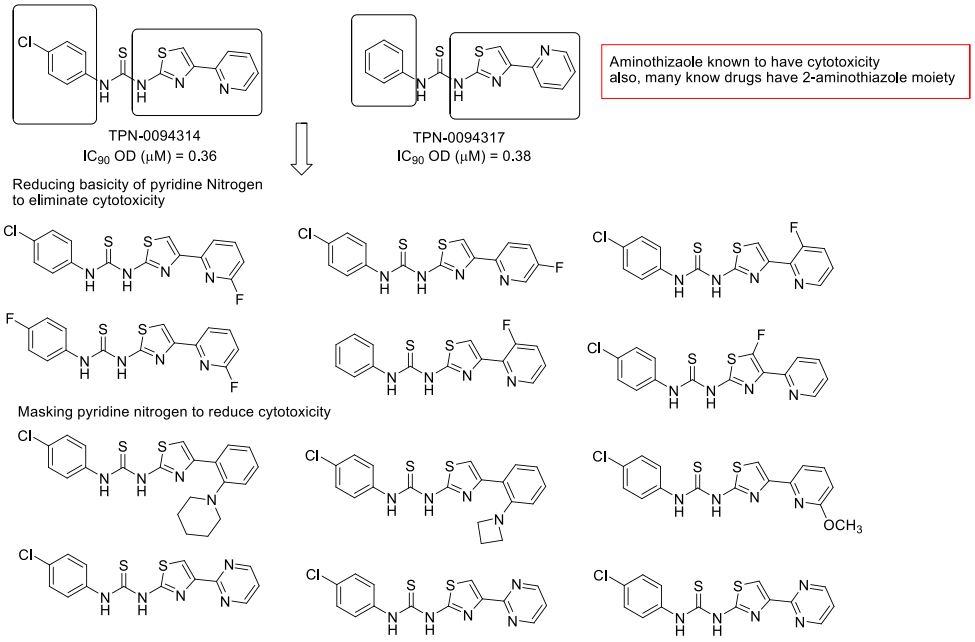


New analogs for hydrazine (PHY) series

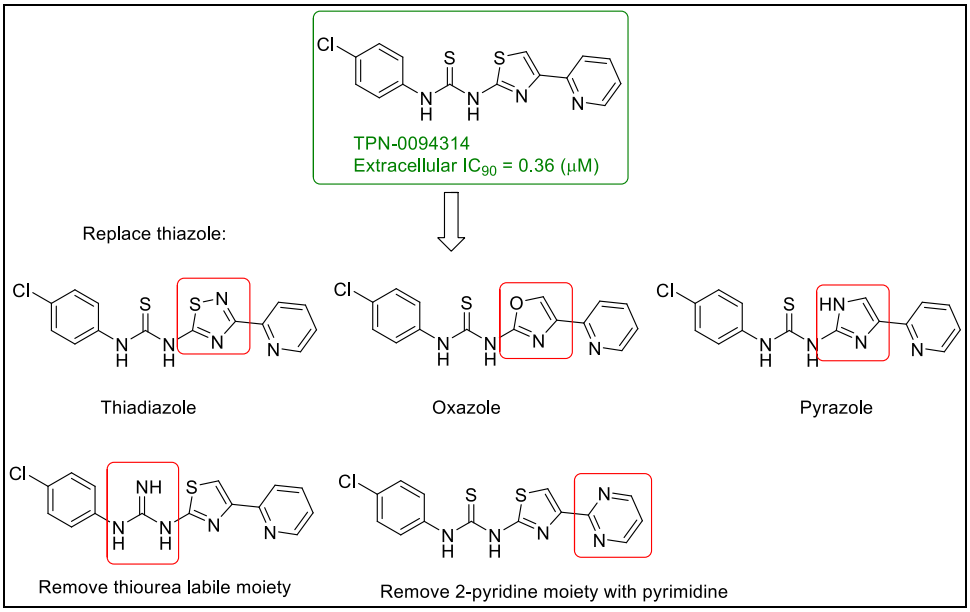
	Anti-tubercular activity			Cytotoxicity			Solid MIC
Compound	MIC (microM)	SD	(n)	IC50 (microM)	SD	(n)	MIC (microM)
TPN-0094309	>100		3	66		4	n/a
TPN-0094310	>100		3	7.3	1.4	2	n/a
TPN-0094311	>100		3	5.1	3.8	4	n/a
TPN-0094312	0.32	0.50	5	0.038	0.0010	4	0.19
TPN-0094313	>100		3	8.9	3.77	4	n/a
TPN-0094314	0.42	0.13	4	0.14	0.00	2	1.6
TPN-0094315	1.8	0.68	4	0.39	0.00	2	1.6
TPN-0094316	>100		3	24	9.9	2	n/a
TPN-0094317	0.35	0.15	5	0.036	0.00	2	1.6
TPN-0094318	>100		3	46	21	4	n/a
TPN-0094319	11		4	0.92	0.25	3	
TPN-0094320	>100		4	>100		3	n/a
TPN-0094321	>100		4	9.2	9.6	2	n/a
TPN-0094322	3.3	0.65	3	0.48	0.19	3	3.1
TPN-0094323	>100		3	>100		3	n/a

Compounds were tested for activity against wild-type *M. tuberculosis.* The minimum inhibitory concentration (MIC) was determined in either liquid medium and defined as reducing growth by >90% after 5 days or on solid medium and defined as reducing growth by >99% after 21 days. Cytotoxicity was determined against HepG2 cells after 72 hours and IC50 defined as concentration leading to loss of 50% viability.

To further address cytotoxicity, we have designed new analogs to modify three zones of the molecule. On the left, we plan to include substitutions with fluorine or chlorine, as well as introducing small-saturated rings or groups such as cyclopropyl, cyclobutyl, isopropyl and methyl groups to balance the lipophilicity of the molecule. On the right hand side, we will focus on attenuating the basicity of the pyridine nitrogen, either by addition of fluorine atom(s) or by addition of amino-pyridine with saturated rings or an electron-rich methoxy group.

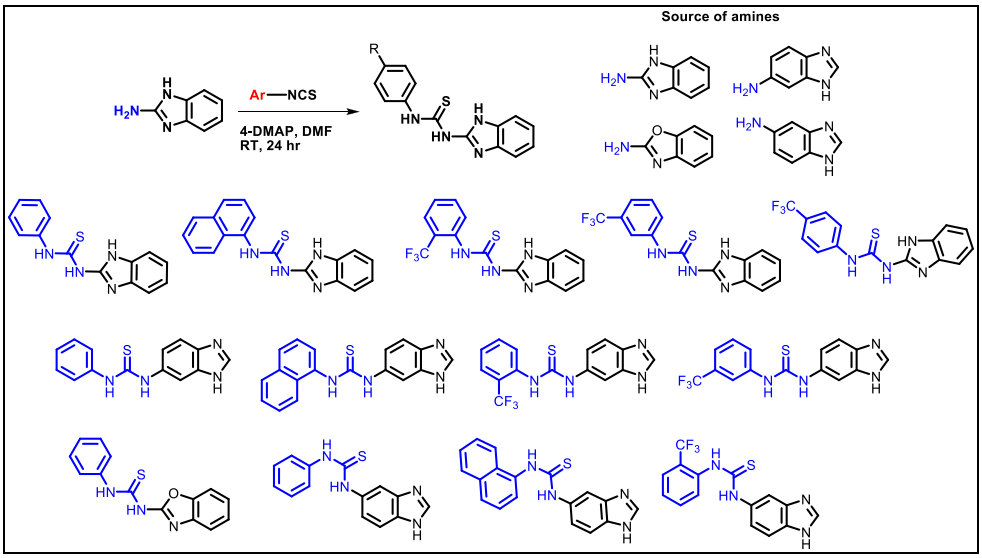


In the center zone we will focus on replacing the thiazole core with other five-membered, rings such as a 1,3,5-thiadiazole, an oxazole or a pyrazole ring. Lastly, we will replace the thiourea with a guanidine moiety to eliminate cytotoxicity.



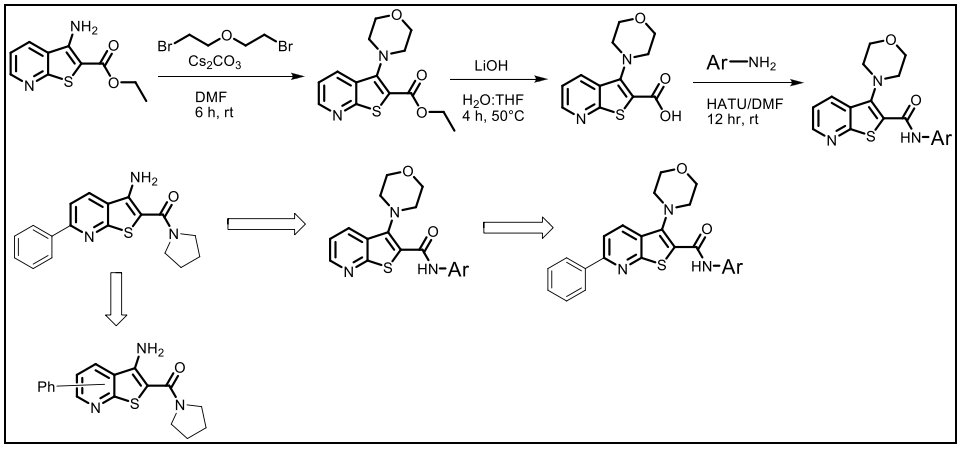
BET and PTU series

We have designed analogs for the BET and PTU series to improve potency and ADME properties by exploring different groups on the either sides of thiourea. We predict that having variety of substituted phenyl rings would improve potency. A simple synthetic scheme is proposed to react isothiocyanates and bicyclic heteroaromatic amines.



TPA design

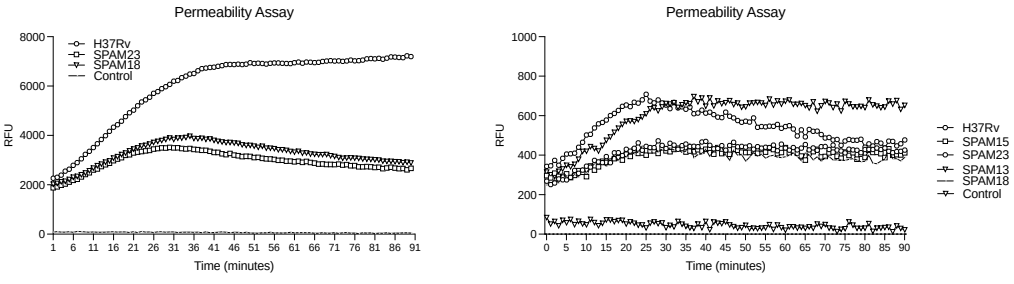
We have designed new analogs for the TPA series and plan to prepare ~15 analogs with a variety of amides or a modified 3-amine on the morpholine. We also designed compounds to explore substitution on the thieno[2,3-b]pyridine ring at the different positions.



Specific Aim 2. Determine the mechanism of kill induced by LepB inhibitors.
The development of novel TB drugs that shorten therapy is a key objective for our overall program, but we do not yet understand how anti-tubercular agents kill the bacteria. Aim 2 is to determine how inhibition of LepB leads to rapid cell death. We will explore several avenues. We will determine if cell death results from protein accumulation in the membrane and/or loss of specific secreted proteins, as well as the effects on cell wall integrity and permeability. We will monitor bacterial viability under conditions relevant to infection during LepB inhibition and determine why LepB inhibitors are more active against non-replicating bacteria.

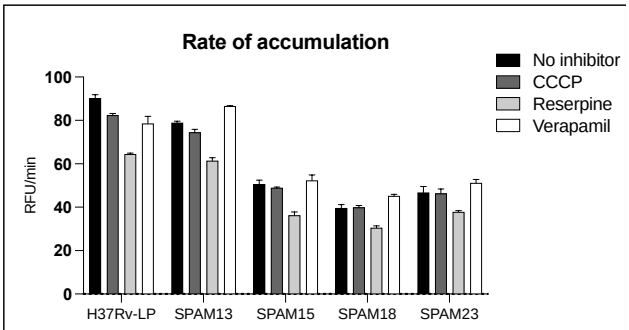
2.1 Determine if cell death is induced by accumulation of proteins in the membrane:
We have generated plasmids containing a non-cleavable signal peptide fused to Gfp in order to determine if accumulation of proteins in the membrane leads to loss of viability. We have transformed plasmids into *M. tuberculosis*.

2.2 Determine if LepB inhibition changes cell wall structure and/or permeability
Inhibition of LepB could have major impacts on the cell wall structure. We measured ethidium bromide uptake to determine if there are changes in cell permeability. We used recombinant strains of LepB with lowered levels of expression. Strains were grown to log phase (OD ~0.8), washed and resuspended in PBS plus ethidium bromide. Uptake was monitored over 90 minutes by fluorescence. Our initial data showed that the wild-type strain accumulated more ethidium bromide over time and at a faster rate. We repeated the study with additional strains and confirmed these results.



M. tuberculosis recombinant strains were grown to log phase, washed and resuspended in PBS. Accumulation of ethidium bromide was monitored by fluorescence in 96-well plates. Data are average of duplicate or triplicate wells.

Accumulation of ethidium bromide could be due to changes in cell wall permeability or changes in efflux. In order to determine whether efflux was changed in the LepB under expressing strains we monitored ethidium bromide uptake in the presence of efflux inhibitors. Again we saw reduced uptake of ethidium bromide in three of the under-expressing strains. A small change in uptake was noted with reserpine, but this reduced the overall uptake rate, rather than increasing it. Therefore we concluded that the change is not due to increased efflux, but rather changes in the cell wall.



M. tuberculosis recombinant strains were grown to log phase, washed and resuspended in PBS. Accumulation of ethidium bromide was monitored by fluorescence in 96-well plates. Data are reported as rate of accumulation per minute (duplicate wells).

We also measured the activity of cell wall inhibitors against the recombinant strains; there was no difference in the MIC for isoniazid or ethambutol for any of the strains.

Specific Aim 3. Determine the role of $LepB_{Mtb}$ under conditions relevant to infection and identify the key residues required for proper $LepB_{Mtb}$ function.

LepB is an attractive drug target, but we do not yet understand its function during infection. We hypothesize that LepB is essential in all environments due to its critical role in protein secretion and function, but the LepB-dependent essential secretome may vary under different conditions. Therefore we will look at the consequences of LepB under-expression in terms of altered protein secretion and survival in response to stresses relevant to infection. *Mtb* LepB has a number of unusual features including an additional cytoplasmic domain and six cysteine residues located in motifs associated with redox-sensitive proteins. We will determine if these are essential amino acids and if they play a role in protein function.

3.2 Determine the role of cysteine resides in LepB function

LepB has some unusual features in *M. tuberculosis,* including the presence of multiple cysteine residues which could be involved in redox functions. In order to determine the role of the cysteine residues, we generated plasmids carrying mutant alleles with the cysteine residues modified (to alanine). We used gene switching to determine which alleles were functional in *M. tuberculosis*. Recombinant strains were verified by sequencing. Our results demonstrate that 5 of the 6 cysteines are not required for LepB function in live cells, since strains were viable carrying LepB alleles with the following mutations: C104A, C107A, C218A, C250A and C254A. No differences in growth kinetics were seen for any of the mutant strains. We were unable to generate viable strains with the $LepB_{C186A}$ allele, confirming that this residue is critical for LepB function. We have generated culture filtrates from each strain grown in protein-free Sauton's medium to determine if there are any gross changes in the secretome

What opportunities for training and professional development has the project provided?
How were the results disseminated to communities of interest?

Dr Talukder received training in medicinal chemistry and drug discovery from Dr Sultan Chowdhury. Dr Harding received training in microbiology, drug discovery and molecular biology from Professor Tanya Parish. Mr Hardy received training in microbiology, drug discovery and molecular biology from Professor Tanya Parish and Ms Stephanie Anover-Sombke. Mr Pham received training for work under Biosafety Level 3 from Professor Tanya Parish; Dr Harding received training for work under Biosafety Level from Dr Arielle Butts. All staff are receiving Professional Development by attending internal and external seminars and journal clubs.

We aim to complete the following objectives:

- Determine the target for each series
    - Determine whether the compound series target secretion directly using a reporter assay for secretion.
    - Generate and sequence resistant mutants.
    - Complete the over-expression library screen.
- Determine the pharmacophore
    - Complete the MICs for all active compounds against the wild-type strain and the LepB-UE strain.
- Evaluate all series potential for lead generation
    - Complete kill kinetics for series under replicating/nonreplicating conditions
- Determine the mechanism of kill induced by LepB inhibitors.
    - Complete studies using the non-cleavable signal peptide fused to Gfp.
    - Complete permeability studies
    - Complete combination studies with cell wall inhibitors
- Determine why cell death is more rapid under non-replicating conditions
    - Measure LepB expression under different conditions
- Determine if cell death kinetics vary under other conditions
    - Characterize survival of new CRISPRi knockdown strains
    - Determine kill kinetics of molecules in hypoxia and at low pH
- Examine the function of the LepB protein
    - Determine the species–specificity for LepB
    - Determine if LepB expression is conditionally essential using CRISPRi knockdown strains.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change
Nothing to report. No changes in approach.

Actual or anticipated problems or delays and actions or plans to resolve them
There were a number of unanticipated challenges during the past year that slowed down our progress. The grant transfer took longer than anticipated and was completed in October 2020. This resulted in a 11 month halt to work from Nov 2019 to Oct 2020. Theref,ore August 2021 is month 12 of experimental work. Due to the length of time it took to transfer grant, we had to hire new staff for the project. This was challenging during the Covid-19 pandemic restrictions imposed at state and national level. We were able to recruit two key staff members, but we delayed recruiting junior staff due to capacity limits for laboratory work.

Work has been negatively impacted by the Covid-19 pandemic on multiple fronts. Restrictions were placed on our ability to work due to Washington state mandated lockdowns, as well as internal policies from Seattle Children's Hospital. Our laboratories were placed under lockdown with no onsite work allowed (except directly related to COVID-19) from 19 March to 11 May 2020. From 11 May to 8 June 2020 we were allowed onsite at 25% capacity for essential work, from 8 June to 26 May 2021 we were allowed on site at 50% capacity for essential work. We are working at onsite without formal capacity limits, but we are still working under restrictions imposed by our local and state governments, as well as Seattle Children's requirements for safe work; these include physical distancing, vaccination, and masking.

Supplies have been impacted by manufacturing shortages and an increase in demand. Plasticware including filter tips and roller bottles, robotics plasticware including tips and multi-well plates have been difficult to obtain. There continue to be shortages of key supplies for BSL3 work (PPE and plastics), as well as key supplies for microbiology including 96/384-well plates, filter tips and robotics tips.

We do not anticipate these challenges reoccurring, although some of the pandemic-related restrictions are still in place and we do not have a date for when we can return to "normal". We have adapted our working practices and anticipate being able to deliver on the grant milestones, assuming no additional restrictions are imposed. In the next 12 months, we anticipate ramping up work significantly enabling us to get back on track for our milestones.

Changes that had a significant impact on expenditures

Our overall budget was underspent in the last year. This was largely due to the consequences of the pandemic and restrictions placed on travel, on site activity and supplies shortage. We spent less on lab supplies than anticipated, due to reduced work in the labs, as well as long delays in receiving shipments and supplier shortages. However, we anticipate that this shortfall will be used as we accelerate our work in the next year. We have a substantial amount of plasticware on order and thus committed, but not spent (some items on backorder for >3 month). We anticipate increased supply costs in the next year due to this backlog.

Due to the length of time it took to transfer the grant, we had to hire new staff for the project. This was challenging during the Covid-19 pandemic restrictions imposed at state and national level. We were able to recruit two key staff members, but we delayed recruiting junior staff due to capacity limits for laboratory work.

A number of preventative maintenance and service visits were delayed due to shortages of vendor technical support staff as well as restrictions on travel and visitors. Service personnel for robotic equipment have been unavailable to travel and parts have been unavailable with long delays to servicing and routine maintenance resulting in sporadic machinery outages. We have preventative maintenance planned and committed, but not yet spent.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

Publications, conference papers, and presentations

Nothing to report.

Journal publications.

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Reporting Period: 10/01/2020 – 7/31/2021

Name:	Dr. Tanya Parish
Project Role:	PI
Researcher Identifier:	
Nearest person month worked:	1.7
Contribution to Project:	Project oversight and intellectual input.
Funding Support:	N/A

Name:	Christopher Harding
Project Role:	Post-doctoral Researcher
Researcher Identifier:	
Nearest person month worked:	7.2
Contribution to Project:	Biological studies, including assays with M. tuberculosis
Funding Support:	N/A

Name:	Muktadir Talukder
Project Role:	Post-doctoral Researcher
Researcher Identifier:	
Nearest person month worked:	4.3
Contribution to Project:	Design, synthesis and analysis of new analogs
Funding Support:	N/A

Name:	Karen Tam
Project Role:	Research Associate
Researcher Identifier:	
Nearest person month worked:	2.5
Contribution to Project:	Assisting with chemical analysis and preparation and biological studies including assays with M. tuberculosis
Funding Support:	N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

OTHER SUPPORT

PARISH, Tanya

ACTIVE SUPPORT

1 R01 AI090048 (Garneau-Tsodikova) 7/01/2017 – 06/30/2022 0.6 Calendar
NIH/NIAID Total costs
Novel aminoglycoside adjuvants and stand-alone agents to combat tuberculosis
The goal of this project is to develop new adjunct agents to improve the activity of aminoglycosides
Role: Subaward Principal Investigator

1 R03 AI151532-02 (Johnson/Parish Co-PIs) 03/10/2020-02/28/2022 0.12 Calendar
NIH/NIAID
Identifying GroEL inhibitors with activity against replicating and non-replicating *Mycobacterium tuberculosis*
Role: Co-Principal Investigator

INV-005585 05/01/2020 – 04/30/2023 2.2 Calendar
Bill & Melinda Gates Foundation
Identification of TB drug candidate(s) with a novel mode of action
Role: Principal Investigator

5 R01 AI129360-03 (Parish) 09/01/2018 – 08/31/2023 2.4 Calendar
NIH/NIAID
The role of Esx-3 in mediating drug resistance
The aims of this project are to determine the role of metal ions in the mode of action of the AmT, 8HQ, and NTS compound series; determine the role of Esx-3 in resistance to multiple compound classes; and determine the intracellular targets of the 8HQ and NTS classes
Role: Principal Investigator

W81XWH1910321 (Parish) 08/01/2019-07/30/2023 2.4 Calendar
Dept. of the Army -- USAMRAA
Protein Secretion as a Novel Drug Target for Tuberculosis
The specific aims for this project are to develop a pharmacophore model for LepB inhibitors and generate lead compounds, determine the mechanism of kill induced by LepB inhibitors, and to determine the role of $LepB_{Mtb}$ under conditions relevant to infection and identify the key residues required for proper $LepB_{Mtb}$ function.
Role: Principal Investigator

5 R01 AI132634-03 (Parish) 07/13/17-06/30/2022 1.8 calendar
NIH/NIAID
High content screening of *Mycobacterium tuberculosis*
The aim is to develop a novel high content screen to identify compounds that act against *M. tuberculosis* inside macrophages.
Role: Principal Investigator

W81XWH12010737 (Parish) 09/01/2020-08/31/2023 1.2 Calendar
Dept. of the Army -- USAMRAA
Morpholinobenzamides as potent anti-tubercular agents targeting mycobacterial respiration
Morpholinobenzamides as potent anti-tubercular agents targeting mycobacterial respiration. The specific aims for this project are to optimize drug-like properties of the compound and demonstrate efficacy in an animal model of infection and to investigate combination therapy with other respiratory inhibitors.
Role: Principal Investigator

INV-001599 (Pogliano) 5/18/2020-7/31/2021 0.12 Calendar
Bill & Melinda Gates Foundation
Mode of action platform for Mtb
To develop platform technology for predicting inhibition of specific mycobacterial cellular processes/pathways by inhibitors
Role: Investigator

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report.

9. APPENDICES:

Nothing to report.