Design, Synthesis and Biological Evaluation of Novel Antimycobacterial Acetamides

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INTRODUCTION

Tuberculosis remains as part of the top ten leading causes of global deaths per year, even with a known and defined treatment plan. Tuberculosis is a multifaceted issue due to socio/political, economical, and environmental issues that is unique to the region it infects. Mycobacteria falls under two categories: tuberculosis and nontuberculous (NTM). The distinction is important because the available drug therapies only show efficacy in only *Mycobacterium tuberculosis* and not nontuberculous causing bacteria. NTM is an opportunistic pathogen and typically infects people with compromised immune systems such as HIV, COPD, cystic fibrosis, and bronchiectasis.[3] Therefore, it is imperative for novel compounds to be designed and developed for the pharmaceutical pipeline.

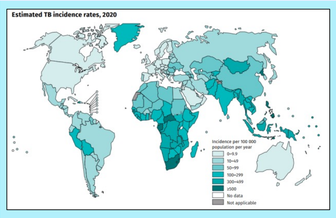


Figure 1. World Health Organization estimation of global TB burden.

Currently, the drug regimen for TB patients is an antibiotic cocktail composed of four drugs: isoniazid, rifampicin, pyrazinamide, and ethambutol and treatment time is a minimum of six months (Figure 2). During the first two months of treatment, all four drugs are used, and the following four months use only isoniazid and rifampicin. And oftentimes, a six-month course of treatment goes longer because of bacterial resistance, patient compliance, and accessibility to drugs and doctors.[3,4] The prolonged treatment often leaves patients with irreversible hearing loss and leaves their immune system compromised from overuse of antibiotics.

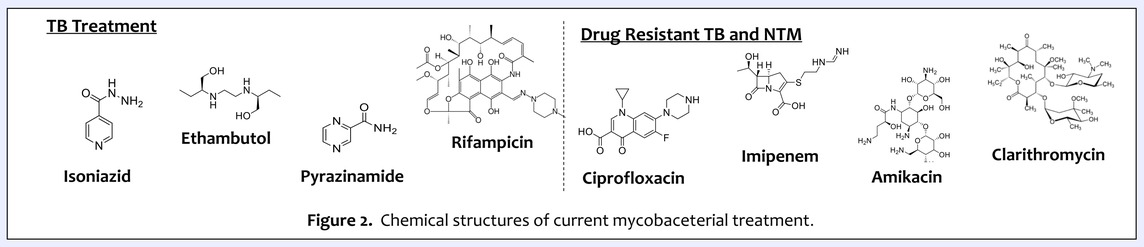


Figure 2. Chemical structures of current mycobaceterial treatment.

Another added complication is the diagnosing process of TB and NTM, since both types of mycobacterial infections show up positive for the conventional smear acid fast staining method, which then leads to NTM being underestimated in patients. There is still no efficacious treatment for NTM patients, but they are prescribed an eighteen-month treatment cycle of the same drugs (**Figure 2**) used for TB and with a twelve-month sputum negative period before being classified as being no longer affected by this infection.[3] The acetamides are a promising series because of their structural activity in remaining potent to *M. tb*, *M. avium* and *M. abscessus*, without showing evidence of ototoxicity.

ACETAMIDE DESIGN

The IC pharmacophore requirements are 1) a bulky aliphatic ring (R1), 2) two H-bond donors (NH groups), 3) H-bond acceptor (O) and 4) an aromatic ring (indole). These requirements ensure antimycobacterial activity. The IC class of compounds are, despite their high potency, are poorly soluble (<1 µg/mL) in physiologically relevant aqueous environments. This will surely limit their translational potential as poor aqueous solubility will limit IV administration options, reduces drug absorption and can lead to significant drug-drug interactions. Therefore, we have designed a more polar acetamide-based class of antimycobacterials over the IC class, and yet retains the required pharmacophore, ensuring antimycobacterial activity. This was accomplished by reducing the number of lipophilic atoms by deleting the CH at the 3-position on the indole ring (**Figure 3**).

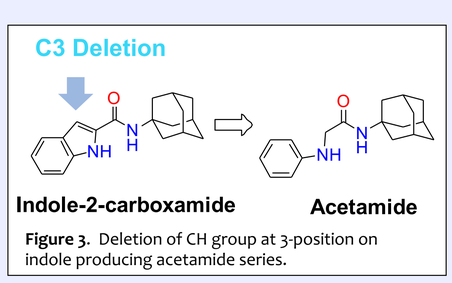
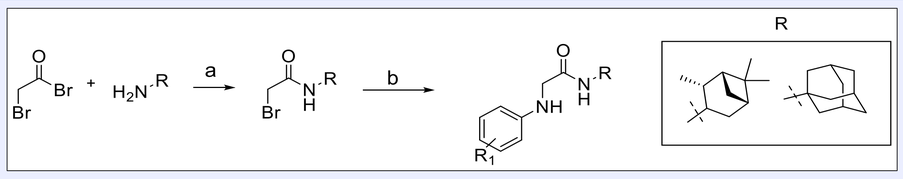


Figure 3. Deletion of CH group at 3-position on indole producing acetamide series.

ACETAMIDE SYNTHESIS



Scheme 1: Synthetic pathway for the production of acetamide series. Reagents and conditions: (a) Triethylamine, 0°C, 4 h; (b) Aniline analogs, TEA, THF, 60° Celsius; overnight Work up with ethyl acetate and water, purification by flash chromatography; % yield **N200**: 22%, **N192**: 46%. Acetamides were characterized with ^{1}H NMR, ^{13}C NMR, mass spectrometry, and HPLC.

In vitro ADMETox

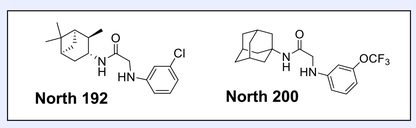


Table 1. Antimycobacterial activity of para-substituted acetamide. MIC units are in µg/mL. Kinetic solubility and human plasma protein binding values for acetamide with MIC values ≤1 µg/mL against *M. tb* or *M. abscessus*.

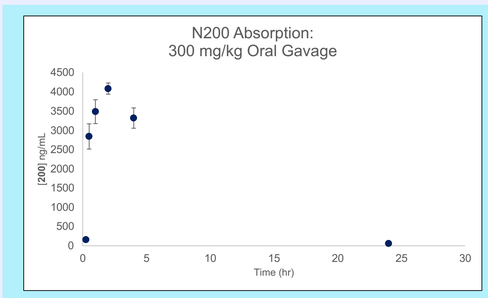
North #	MIC (µg/mL)		ADME and Toxicity				
	M. abscessus ATCC 19977	*M. tb* mc2 6206	Solubility[a] (µg/mL)	Permeability[b] (x10^{-6} cm/s)	HPPB[c] (% Bound)	Metabolic Stability[d] ($t_{1/2}$ = hr)	Toxicity[e] IC_{50} (µg/mL)
192	**0.5**	**0.25**	16.3 ± 0.3	1.33 ± 0.01	94.1 ± 0.02	5.3±0.2	>20
200	**0.5**	**0.25**	20.5 ± 2.4	3.61±0.04	94.9 ± 0.3	0.7±0.0	>20

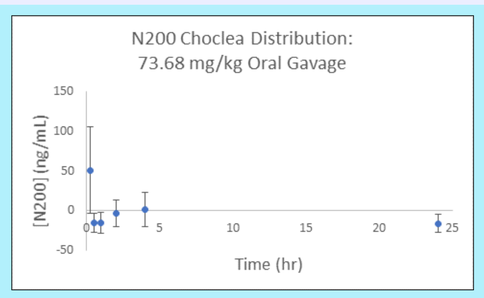
[a]Kinetic solubility assay
[B]Parrallel artificial membrane permeability assay (PAMPA)
[c]Human plasma protein binding (HPPB)
[d]Metabilic stability against human S9 fraction
[e]Cytoxicity was determined against WI-26 VA4 lung cell line

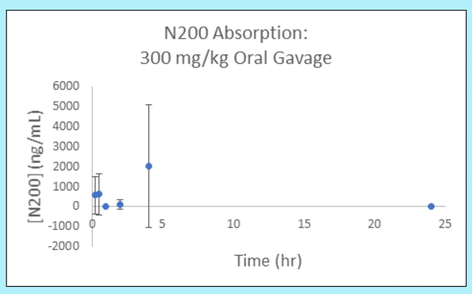
In vivo PHARMACOKINETICS

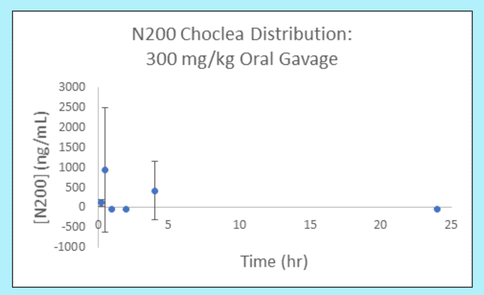
We carried a comparative pharmacokinetic (PK) study on **N200** to determine the effective dose for *in vivo* efficacy trials. A single dose of 73.68 and 300 mg kg^{-1} was administered orally (P.O.) to male and female C57BL6 mice. Compound quantification from blood plasma and cochlea tissue occurred at the 0.25, 0.5, 1, 2, 4, and 24 hr time points.

Table 2. Graphs are shown for **N200** with a comparison of low dosage and high dosage.









N200 IS NONTOXIC IN ZEBRAFISH MODELS

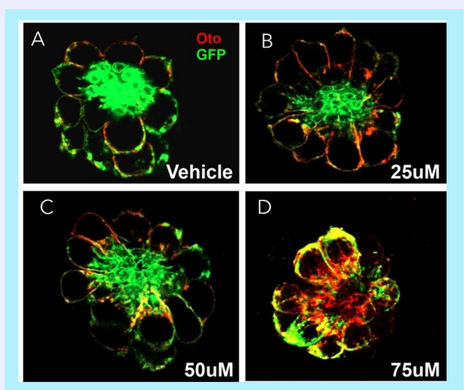


Figure 4: Increasing concentrations of **N200** do not affect the integrity or survival of neuromast HCs *in vivo*. **A.** Vehicle-only treated (control) neuromast; **B-D.** neuromasts treated with 25, 50 and 75 uM, respectively, of **N200** in swimming water overnight. Regardless of the drug concentration tested, no structural or changes were observed on HCs or neuromast anatomy.

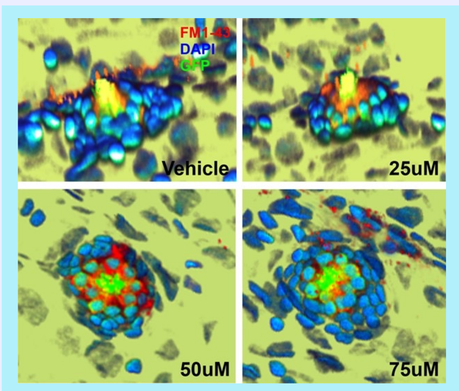


Figure 5: Mechanotransduction channels are at the core of sensory HCs' activity. The styryl pyridinium dye FM1-43 provides an optical measure of ion channel function and therefore used as a proxy for the viability of the neuromast cells treated with **N200**. Results summarized in this figure show a rapid FM1-43 entry into the HCs, which is comparable between control and experimental groups treated with different concentrations of **N200**.

CONCLUSIONS

The acetamides were scaled up successfully with yields of 22% and 46%. Currently, it has been determined that **North 200** can be rapidly absorbed and eliminated in mouse models. The C_{max} was determined to be 3996.6 ± 2861.2 ng/mL and 2022.5 ± 3053.0 mg/mL for the low and high dose. The T_{max} was achieved at 15 minutes and 4 hours for the low and high dose. It has been observed that very little drug was found in cochlea tissue, suggesting little to no ototoxicity. No structural changes in hair cell anatomy were seen with any concentrations from **N200**. The mechanotransduction channels show that there is rapid entry for both the control and experimental groups containing **N200**. Current directions for this project is working on improved formulation because poor solubility in physiological buffers. Additional bulky aliphatic rings will be synthesized and tested for drug efficacy.

ACKNOWLEDGEMENTS

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