Structure-Activity Relationship of Novel Diphenyl Ureas Targeting *Mycobacterium*

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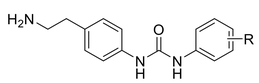
Abstract

In 2017, the World Health Organization reported that 10 million people were infected with tuberculosis, 1.6 million of whom died. Tuberculosis is caused by a bacterium called *Mycobacterium tuberculosis,* which primarily infects an individual's lungs. Unfortunately, failure to adhere to the long and arduous drug regimen has contributed to the emergence of antibiotic-resistant strains of *M. tuberculosis*. Therefore, the need for novel antibiotics is imperative to saving millions of lives. Our lab has recently developed a family of diphenyl ureas that exhibited increased antimicrobial activity toward *Mycobacterium.* Reported herein is the continuation of our previous research involving the synthesis of compounds with increased ester chain lengths and varying substituents on the phenyl ring. Compounds were confirmed using NMR spectroscopy and tested for antimicrobial activity using disk diffusion assays.

Background

- In 2017 alone, an estimated 10 million people were infected with tuberculosis. Of the 10 million infected people, 1.6 million died.
- Tuberculosis is caused by a bacteria called *Mycobacterium tuberculosis.* It is transmitted through aerosol droplets and primarily affects the lungs.
- Current treatments for TB are long and arduous. Affected individuals are prescribed a six-month treatment involving isoniazid, rifampicin, pyrazinamide, and either ethambutol or streptomycin.
- Failure to adhere to current drug treatments has led to antibiotic resistance. Multi-drug resistant (MDR), extensively-drug resistant (XDR), and totally drug resistant (TDR) TB strains have been reported.

Previous Research



- Previously made diphenyl ureas exhibit antimicrobial activity
- Analyzed with disk diffusion tests

Urea	R	Zone (mm)	
		S. aureus	*E. coli*
31	4-bu ester	11.8	9.0
29	--	8.0	8.5
24	3-Cl	8.7	10.5
22	4-nitro	8.0	9.0
21	3-nitro	7.8	33.0
12	4-t-bu	11.3	10.0
11	4-i-pr	6.7	11.2
07	3,4-dimethyl	10.7	9.5

Figure 1. **Previous Data.** Results of disk diffusion tests for diphenyl ureas. Tests were conducted by Kelsie Nauta.

Biological Targets

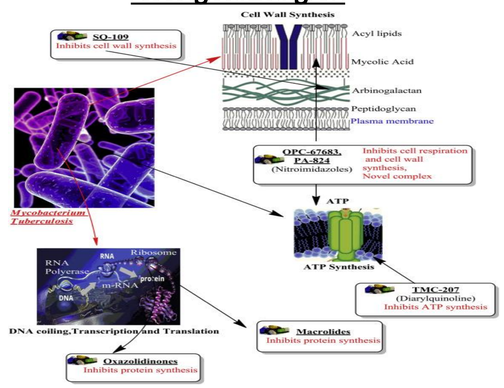


Figure 2. **Biological targets for antibiotics.** Vasava, Mahesh S., et al. "Development of new drug-regimens against multidrug-resistant tuberculosis." *Indian Journal of Tuberculosis*, vol. 66, no. 1, January 2019, pg. 12-19. *Elsevier,* https://doi.org/10.1016/j.ijtb.2018.07.004.

Previous Ester Modifications

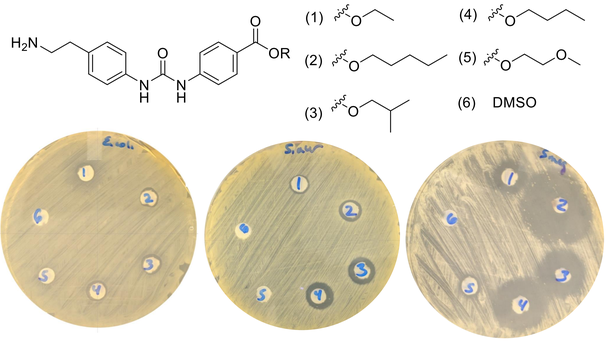
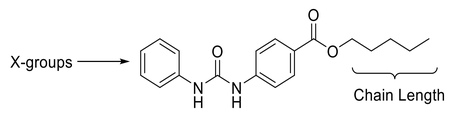


Figure 3: **Ester Derivatives.** Disk diffusion tests for esters vs. *E. coli*, *S. aureus*, and *Mycobacterium* show increased potency with increased chain length on *Mycobacterium*. Work completed by Phil Dietz and Dr. Morgan.

Hypothesis

By increasing the ester chain length and incorporating substituents from our previous study, we can develop a structure-activity relationship for these compounds. We hypothesize that these compounds will be more potent antibiotics against *Mycobacterium*.

Proposed Targets



To test our hypothesis, we will synthesize molecules with increased ester chain lengths and different X groups attached to the phenyl ring.

Synthetic Routes

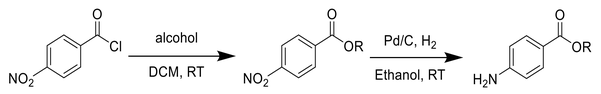
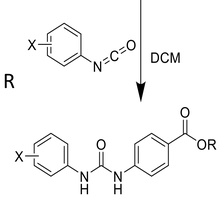
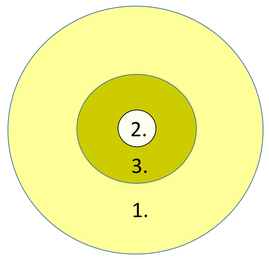


Figure 4. **Overall Synthesis.** The complete three-step synthesis of the diphenyl urea compounds with varying R chain lengths and X groups.

- Structures were confirmed by H-NMR and C-NMR analysis.



Disk Diffusion Test

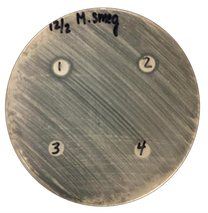


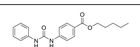
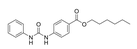
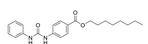
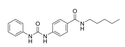
1. A lawn of bacteria is gently swabbed onto an agar plate.
2. A disk that contains the desired compound is added to the plate. Multiple disks can be added to the same plate.
3. The agar plate is incubated for a few days, and the resulting zone of inhibition is measured.

Figure 5. **Disk Diffusion Test.** The concentration of the compound decreases as the distance from the disk increases. Therefore, a larger zone of inhibition indicates a more effective compound since it takes less of the compound to inhibit bacterial growth.

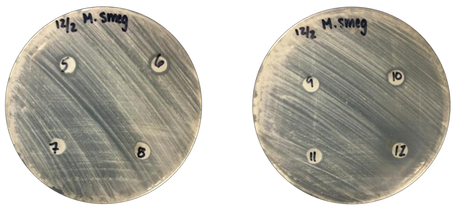
Biological Testing Results for Chain Length

Figure 6. **Biological Testing of Ester Length Compounds.** The results of the disk diffusion test for the compounds with varying ester chain lengths showed little antimicrobial activity against *Mycobacterium*.



Disk Number	Compound	Zone of Inhibition (mm)
1		9.5
2		9.0
3		0.0
4		0.0

Biological Testing Results for X Groups



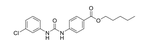
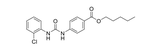
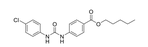
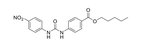
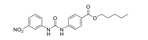
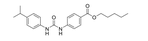
Disk Number	Compound	Zone of Inhibition (mm)
5		9.0
6		9.5
7		0.0
8	DMSO	0.0
9		7.5
10		8.0
11		7.5
12	Oregano Oil	11.5

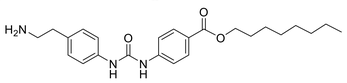
Figure 7. **Biological Testing of Various X Group Compounds.** DMSO was used as a negative control, and solution of diluted oregano oil was used as a positive control. The results of the disk diffusion test for the compounds with varying X group substituents showed little antimicrobial activity against *Mycobacterium*.

Conclusions

- 10 new urea derivatives were synthesized, 4 of which varied in chain length, and 6 of which varied in X groups and their positions on the phenyl ring.
- Without the ethyl amine group from the initial studies, little antimicrobial activity was observed.

Future Directions

- Synthesize compounds with both the ethyl amine and increasing ester chain lengths



- Retest the 30 compounds from the initial screening against *Mycobacterium*
- Examine a new class of compounds with charged side groups

References

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