Synthesis and evaluation of trehalose - Pks13 inhibitor conjugates targeting *Mycobacterium* species

Umesha Subhani S. Kumbalathara A.D.[a], Priscila Cristina Bartolomeu Halicki[b], Kalera Karishma[c], Benjamin Swarts[c], Kyle Rohde[b] and Steven J. Sucheck*[a]

[a]Department of Chemistry & Biochemistry, University of Toledo, Toledo, Ohio 43606, United States.

[b]Division of Immunity and Pathogenesis, College of medicine, University of Central Florida, Florida 32816, United states.

[c]Department of Chemistry and Biochemistry, Central Michigan University, Mount pleasant, Michigan 48859, United states.

* *steve.sucheck@utoledo.edu*

Abstract

❖*Mycobacterium tuberculosis* (*Mtb*) has unique cell wall, which forms a significant permeability barrier to drug transport, primarily made out of Mycolic acids (MAs).

❖In this study we aimed last step of MA biosynthesis by targeting Pks13 pathway and trehalose utilization pathway.

❖This research highlights the importance of Trehalose as a drug delivery method which can enhance compound uptake and efficacy by "Trojan horse" drug delivery strategy.

❖We found that, in some instances, trehalose served to significantly enhance either the antimycobacterial potency or improve selectivity (by reducing toxicity) of the Pks13 inhibitors.

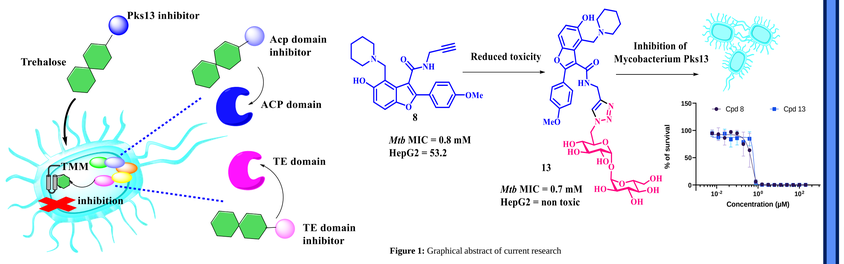


Figure 1: Graphical abstract of current research

Introduction

❖One of the challenges in developing new drugs to target *Mtb* is the unique structure of its cell wall, which includes a distinctive outer membrane known as the mycomembrane.

❖One potential strategy to overcome this permeability barrier involves utilizing endogenous receptors on the bacterial surface that facilitate the uptake of nutrients or cofactors. This approach has shown promise using siderophore-linked compounds using Trojan-Horse drug delivery strategy.

❖In the present study, we envisioned a strategy in which conjugation of a chemotherapeutic for *Mtb*, specifically a Pks13 inhibitor to trehalose could potentially enhance its entry into mycobacteria. This process may be facilitated by the PPE51 transporter,or it may directly access to cytoplasm via the LpqY-SugABC transporter, as illustrated in **Figure 2**.

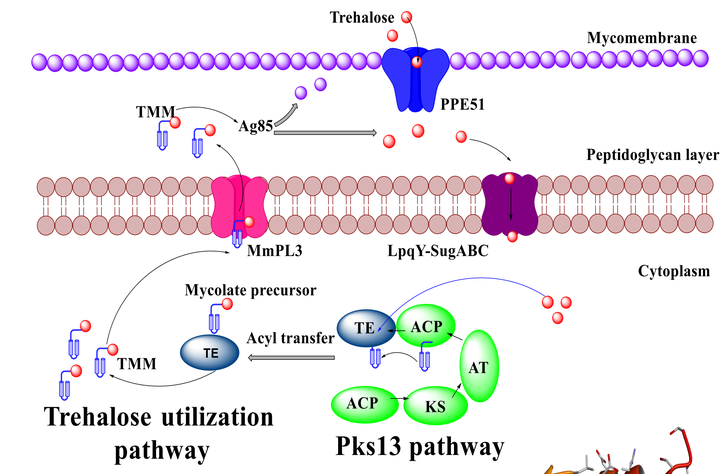


Figure 2: Pks13 and Trehalose utilization pathway

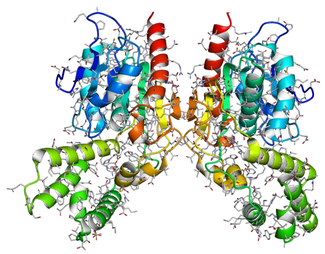


Figure 3: Crystal structure of Thioesterase (TE) domain[3]

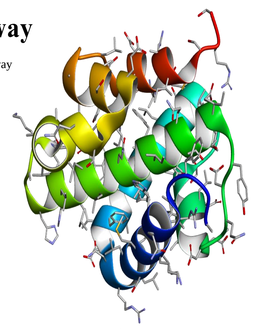
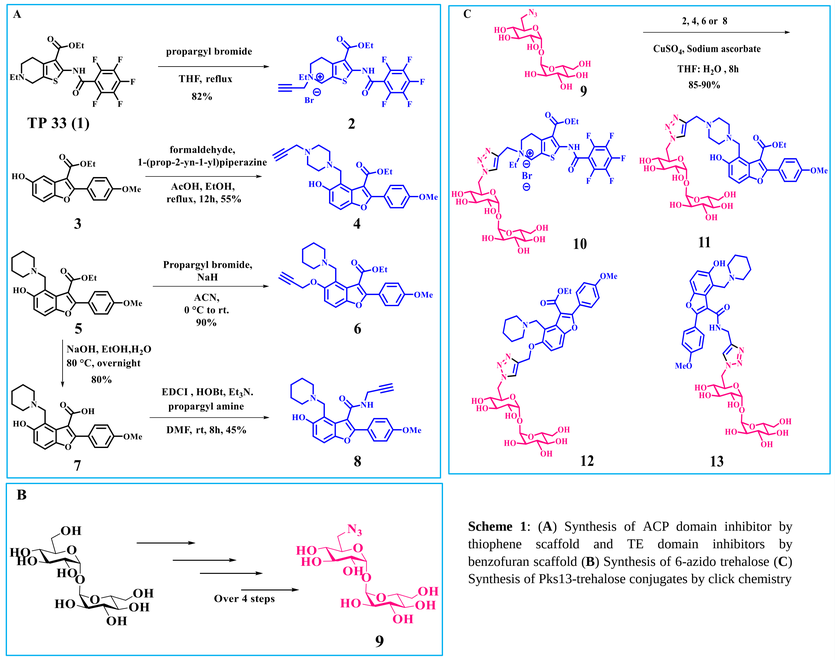


Figure 4: Crystal structure of Acyl carrier protein (ACP) domain[5]

Synthesis of Trehalose conjugated Pks13 inhibitors



Scheme 1: (**A**) Synthesis of ACP domain inhibitor by thiophene scaffold and TE domain inhibitors by benzofuran scaffold (**B**) Synthesis of 6-azido trehalose (**C**) Synthesis of Pks13-trehalose conjugates by click chemistry

HPLC method development and purification

Time (min)	Acetonitrile (0.1% TFA) %	Water (0.1% TFA) %
0-5	5	95
5-10	40	60
10-15	40	60
15-20	100	0
20-25	100	0

Column: RESTEK ultra C8 5µm 150x10.0 mm^2
Mobile phase: Acetonitrile (0.1% TFA) and H_2O (0.1% TFA)
Flow rate: 4 mL/min
Injection volume: 500 µL

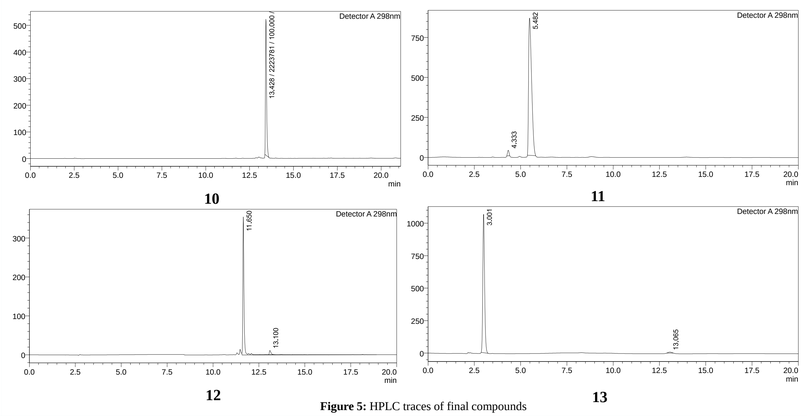
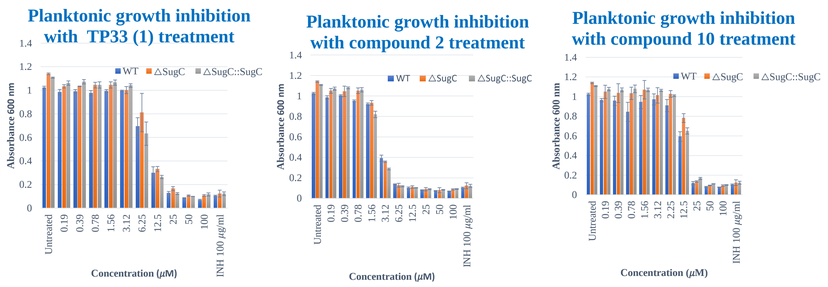


Figure 5: HPLC traces of final compounds

Planktonic growth inhibition Results



Minimum Inhibition Concentration and Cytotoxicity Results

Compound number	MIC (μM)			IC_{50}		SI (*Mtb* CDC 1551)	
	Msmeg mc^2 155	*Mab* 390S	*Mtb* CDC 1551	J774	HepG2	J774	HepG2
2	1.8	1.7	5.8	**NT**	**NT**	>34.6	>34.6
10	8.0	118.6	14.6	**NT**	**NT**	>13.7	>13.7
4	NA	NA	NA	**NT**	**NT**	-	-
11	98.0	200	5.3	**NT**	**NT**	>38.1	>38.1
6	13.0	62.7	1.8	8.19	34.5	4.5	19.0
12	102.2	NA	77.3	**NT**	**NT**	>2.6	>2.6
8	12	NA	0.8	27.7	53.2	33.1	63.3
13	12.6	>200	0.7	**NT**	**NT**	>303	>303
NA= Non-Active				**NT**= Non-Toxic			

Table 1: MIC and cytotoxicity data

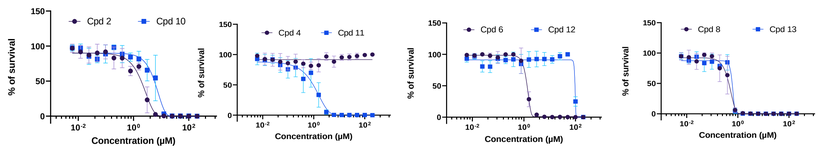


Figure 6: MIC curves for *Mtb*

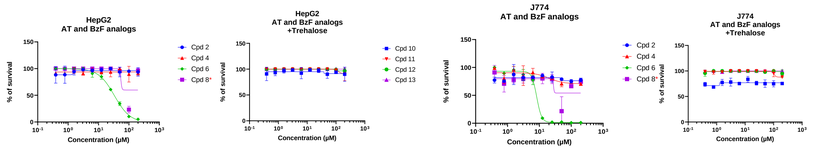


Figure 7: Cytotoxicity dose-response curves

Discussion and conclusion

❖ The goals of this study included **i)** extending the SAR characterization of known Pks13 inhibitors, **ii)** determining the antimycobacterial activity with trehalose conjugates and **iii)** testing a Trojan horse strategy of enhancing compound uptake and efficacy by exploiting endogenous trehalose uptake pathways.

❖ However, trehalose-Pks13 inhibitor conjugate **10**, while active against *Msmeg*, did not rely on import into the cell via the plasma membrane-associated trehalose transporter, LpqY-SugABC.

❖ Interestingly, we discovered compounds that gained antimycobacterial activity or maintained activity while eliminating toxicity upon addition of trehalose (**12,13**). This will lead to new drug development with high efficacy and less off target effect.

References

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Acknowledgement

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