



The 9th International Electronic Conference on Medicinal Chemistry (ECMC 2023)

01–30 November 2023 | Online

Alkyl nitrobenzamides as potential DprE1 inhibitors for the treatment of tuberculosis

Chaired by **Dr. Alfredo Berzal-Herranz**
and **Prof. Dr. Maria Emília Sousa**



pharmaceuticals



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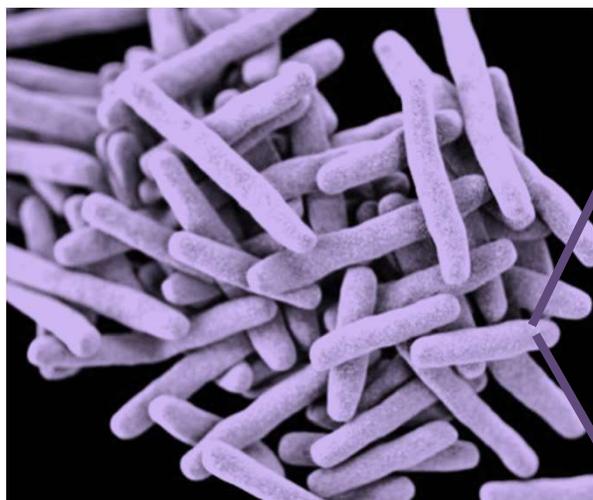


Abstract: Tuberculosis (TB) remains a formidable global health challenge, with an annual reporting of approximately 10 million new cases. The escalating concern revolves around multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB), which present barriers to effective disease control due to their resistance to both first-line and second-line drugs. One of the most promising new targets for TB treatment is the DprE1-DprE2 complex, with ongoing discoveries of novel inhibitors. In prior unrelated research, our group showed that while studying benzoic acid derivatives, the nitro-substituted analogues exhibited interesting activity against *Mycobacterium tuberculosis* (Mtb). This motivated us to synthesize analogous amide derivatives, and our findings show substantial antimycobacterial activity, on par or even greater than known TB drugs. Dinitrobenzamides represent a class of established DprE1 inhibitors, but its alkyl derivatives, akin to the compounds under discussion, were completely overlooked in available literature. Building upon our prior insights, which indicates that 8-carbon atom alkyl derivatives yielded the most potent compounds, we synthesized a series of amide derivatives and, indeed, our study shows 8-carbon atom alkyl amides amongst the most efficacious. This study extensively explores a series of nitro-substituted benzoic amide alkyl derivatives, elucidating the influence of the number and position of nitro-groups on their antitubercular activity. Moreover, we conducted supplementary biological and computational assays to assess the potential targeting of DprE1 by these compounds, as well as their efficacy in an infection model, all of which will be presented and discussed herein.

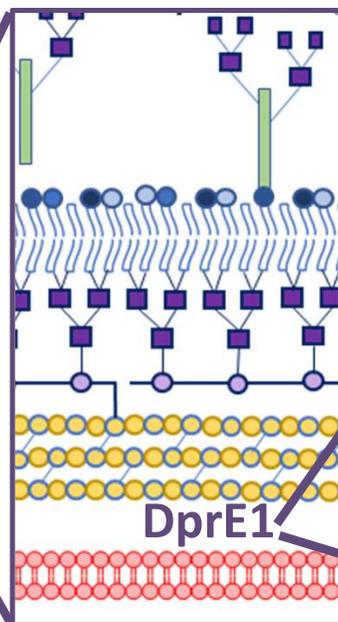
Keywords: Tuberculosis; Nitrobenzamides; DprE1;



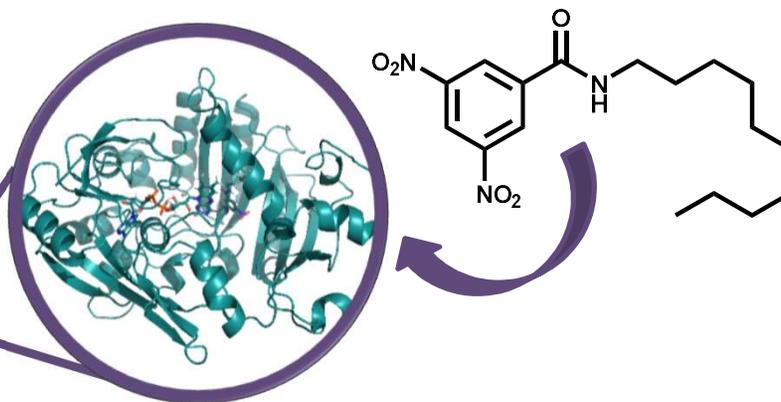
Alkyl nitrobenzamides as potential DprE1 inhibitors for the treatment of tuberculosis



M. tuberculosis



New alkyl dinitrobenzamide
Inhibitors (DNBs)





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Introduction



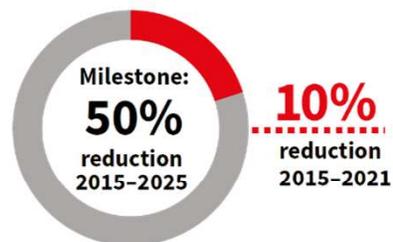
**World Health
Organization**



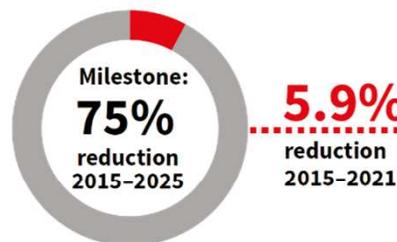
**GLOBAL
TUBERCULOSIS
REPORT
2022**

WHO End TB Strategy: 2025 milestones

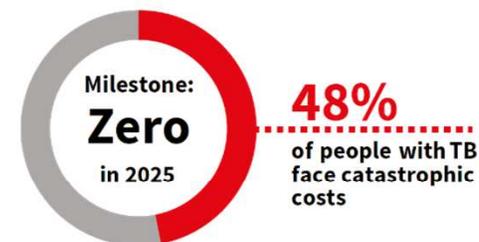
TB INCIDENCE RATE



NUMBER OF TB DEATHS



PERCENTAGE OF PEOPLE WITH TB
FACING CATASTROPHIC COSTS^a



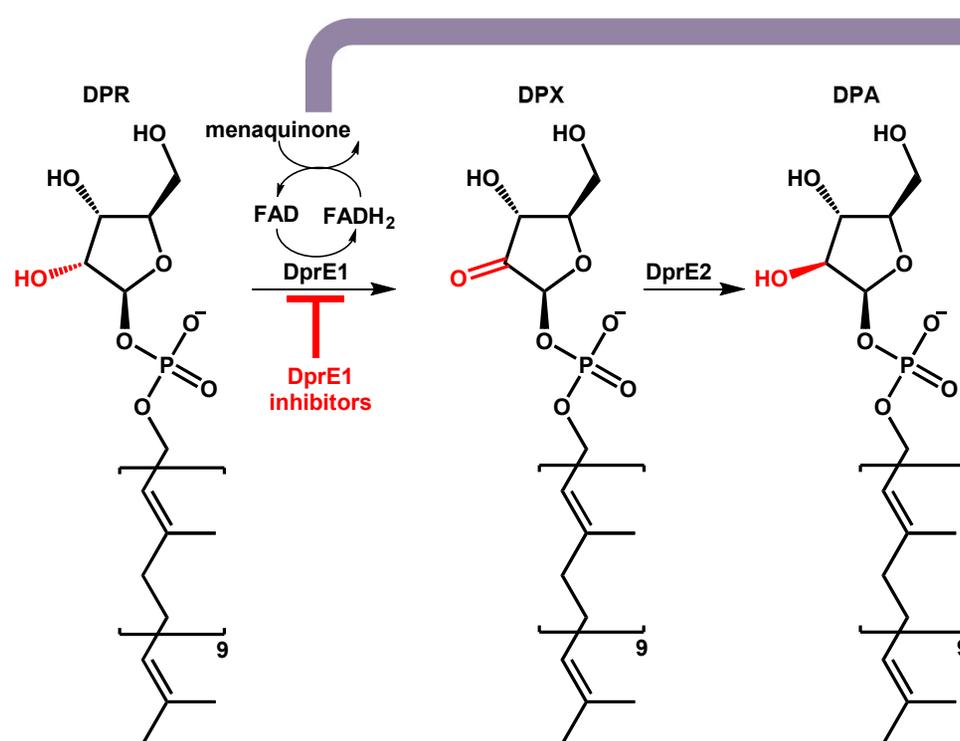
Global tuberculosis report 2022. Geneva: World Health organization; 2022. ISBN 978-92-4-006172-9



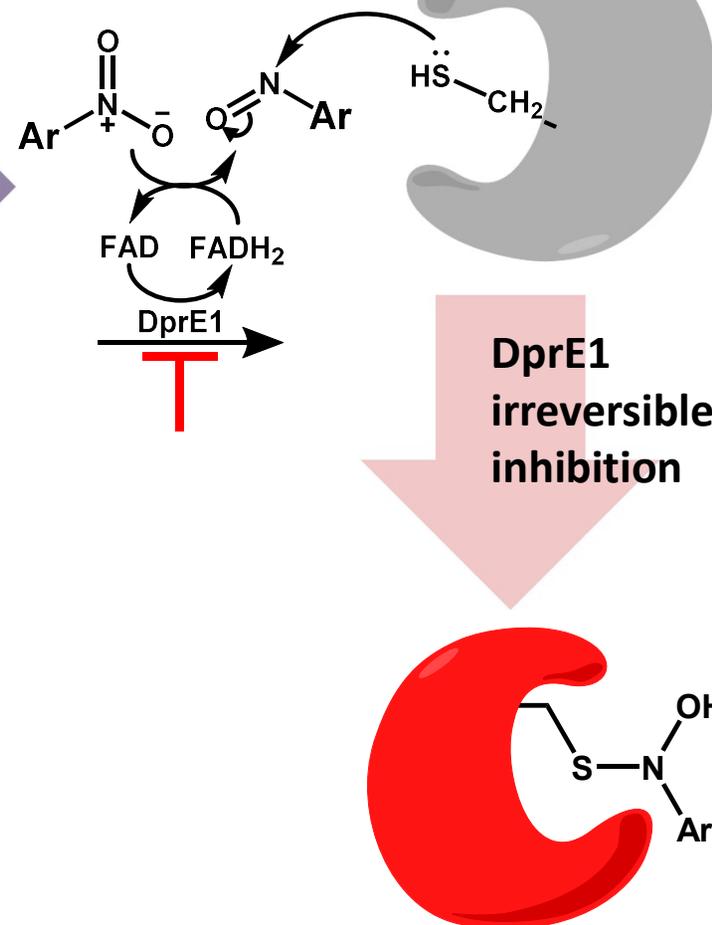
Introduction

Biological targets against TB - Cell Wall Biosynthesis

Decaprenylphosphoryl-beta-D-ribose oxidase (DprE1)



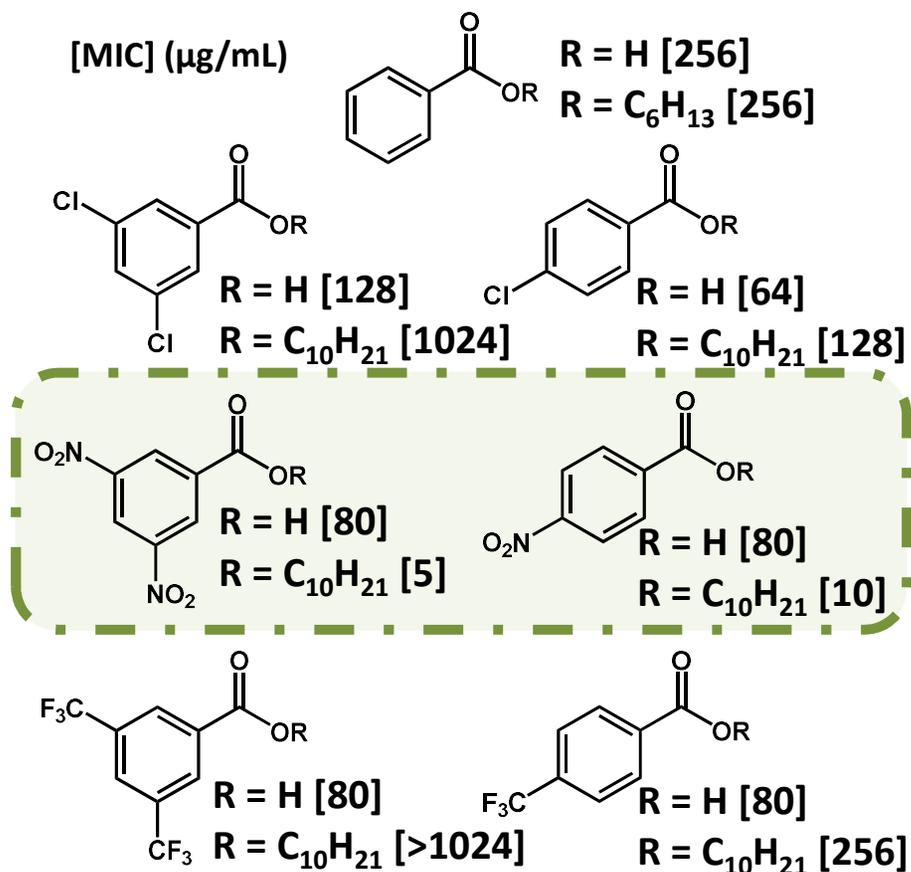
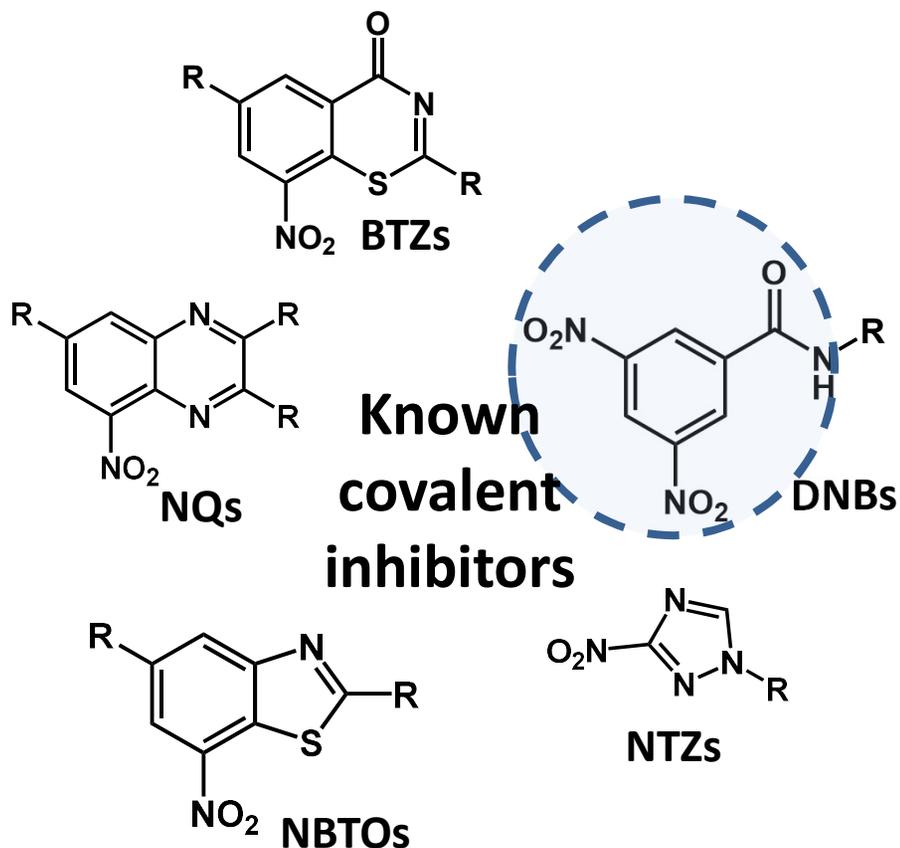
DprE1-DprE2 complex



DprE1
irreversible
inhibition



Past work



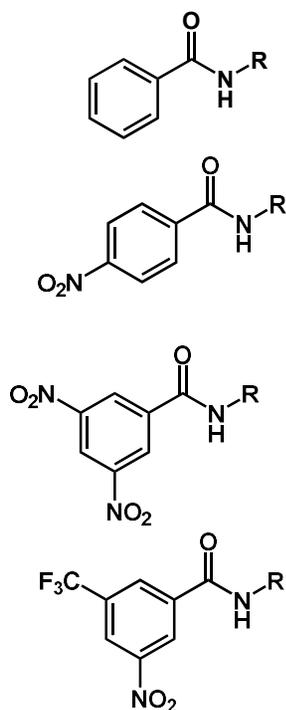
João P. Pais et al, *Microorganisms* **2023**, *11*(4), 969;
doi.org/10.3390/microorganisms11040969



Summary

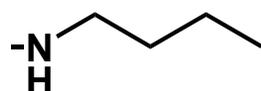
Compound library Synthesis

Nitro-substituted benzamides

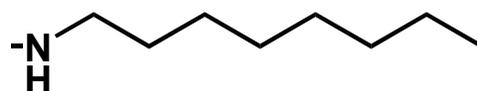


Small, Medium and Long alkyl chain derivatives

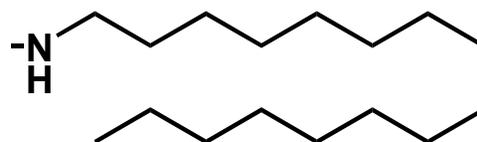
R =



(...)



(...)



Antitubercular activity
screening

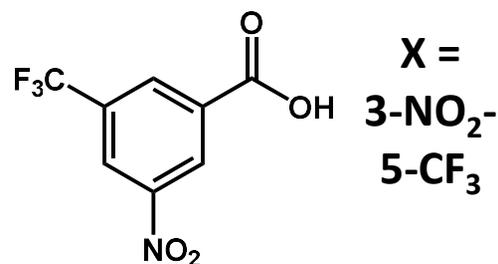
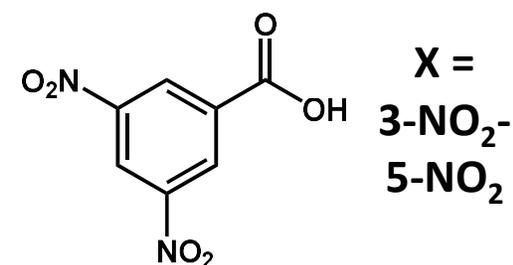
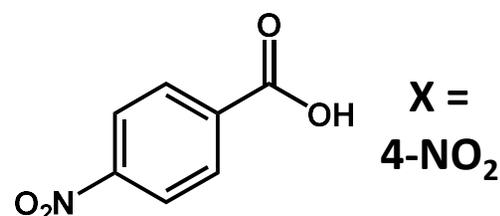
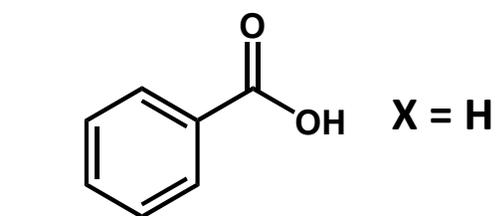
Infection model

Multiple species assay

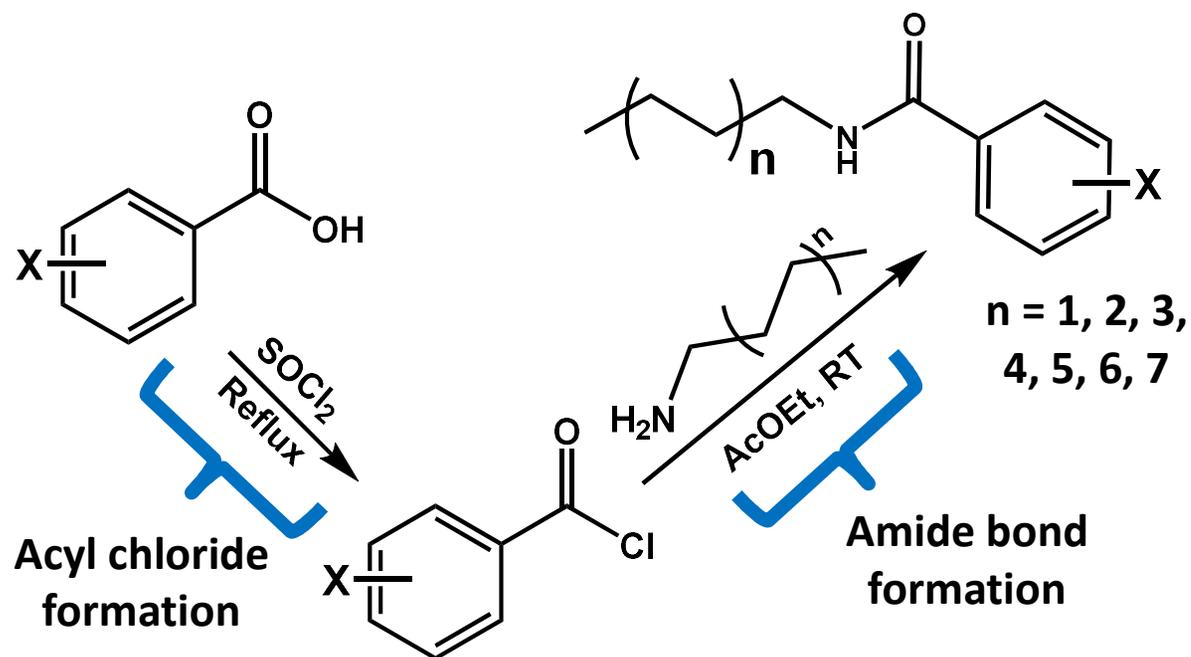
Computational Studies



Results and Discussion: Synthesis



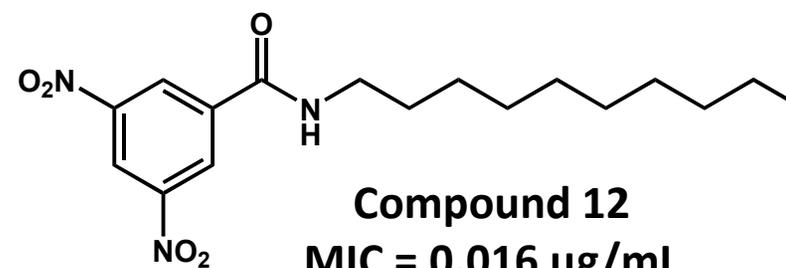
Schotten-Baumann-type reaction





Results and Discussion: Antitubercular activity

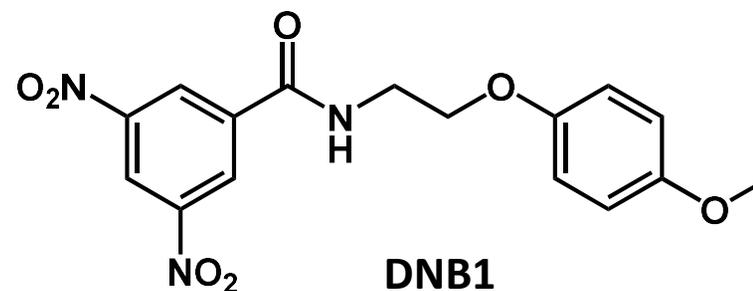
Number	X =	R =	MIC ($\mu\text{g/mL}$)	MBC ($\mu\text{g/mL}$)
1	H	C ₄ H ₉	256	256
2		C ₆ H ₁₃	64	64
3		C ₈ H ₁₇	32	32
4		C ₁₂ H ₂₅	>256	>256
5	4-NO ₂	C ₄ H ₉	256	256
6		C ₆ H ₁₃	32	64
7		C ₈ H ₁₇	128	256
8		C ₁₂ H ₂₅	512	>1024
9	3-NO ₂ -5-NO ₂	C ₄ H ₉	0.5	0.5
10		C ₆ H ₁₃	0.031	0.031
11		C ₈ H ₁₇	0.016	0.031
12		C ₁₀ H ₂₁	0.016	0.016
13	3-NO ₂ -5-CF ₃	C ₁₂ H ₂₅	0.031	0.063
14		C ₁₄ H ₂₉	0.125	0.25
15		C ₁₆ H ₃₁	2	2
16		C ₄ H ₉	2	2
17	3-NO ₂ -5-CF ₃	C ₆ H ₁₃	0,5	0,5
18		C ₈ H ₁₇	0.063	0.063
19		C ₁₀ H ₂₁	0.5	0.5
20		C ₁₂ H ₂₅	0.5	0.5



Compound 12

MIC = 0.016 $\mu\text{g/mL}$

MBC = 0.016 $\mu\text{g/mL}$



DNB1

MIC = 0.016 $\mu\text{g/mL}$

MBC = 0.016 $\mu\text{g/mL}$

**Antitubercular activity
matching the best of known
DNBs!**



Results and Discussion: Infection model

Infection model: Macrophage cells infected with *M. bovis*

mycobacterial cell count (CFUs)
after X days of treatment

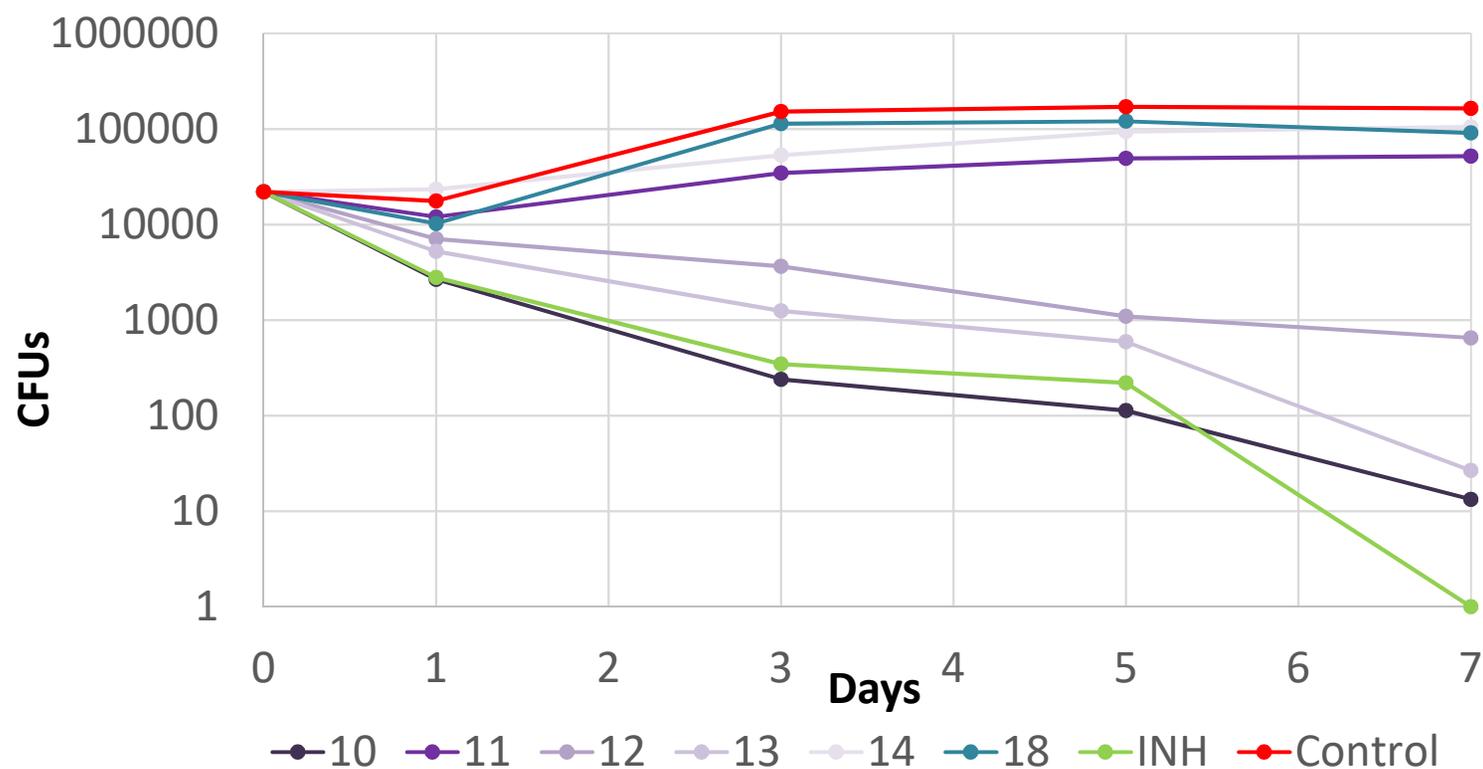
Compounds	Days				
	0	1	3	5	7
10	22000	2667	240	113	13
11	22000	12067	34667	49333	52000
12	22000	7067	3667	1100	653
13	22000	5267	1253	593	27
14	22000	23400	53333	94000	105333
18	22000	10267	114000	120667	91333
INH	22000	2800	347	220	0
Control*	22000	17667	152667	171333	164000

* Infected macrophages without treatment



Results and Discussion: Infection model

Infection model: Macrophage cells infected with *M. bovis*





Results and Discussion: Multiple species assay

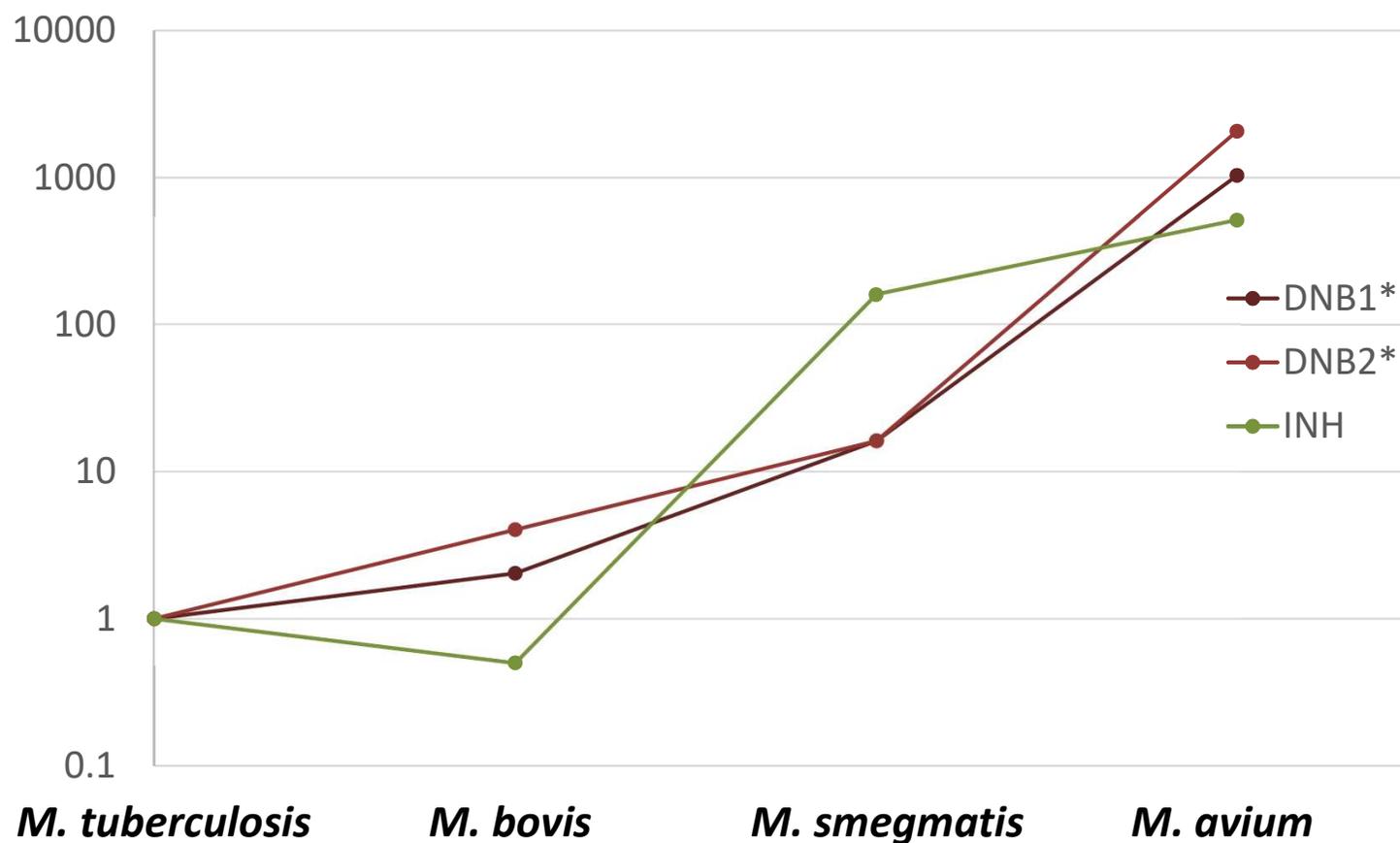
Different bacterial strains have different resistances associated to DprE1 inhibition

Compounds	<i>M. tuberculosis</i>		<i>M. bovis BCG</i>		<i>M. smegmatis</i>		<i>M. avium</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
DNB1*	0.031	0.031	0.063		0.5	1	32	>128
DNB2*	0.031	0.063	0.125		0.5	1	64	>128
INH	0.05	0.05	0.025	0.025	8	>25.6	>25.6	>25.6
4	32	32	32	32	128	128	128	256
9	128	256	128	512	1024	1024	1024	1024
12	0.5	0.5	0.5	1	4	4	64	512
13	0.063	0.063	0.25	0.25	1	1		
14	0.016	0.031	0.016	0.016	0.25	4	512	>512
15	0.016	0.016	0.063	0.063	1	1	>512	>512
16	0.031	0.063	0.083	0.166	0.667	>2,66	>512	>512
17	0.125	0.25	0.25	0.25	1	4		
18	2	2	2	4	32	>64		



Results and Discussion: Multiple-strains assay

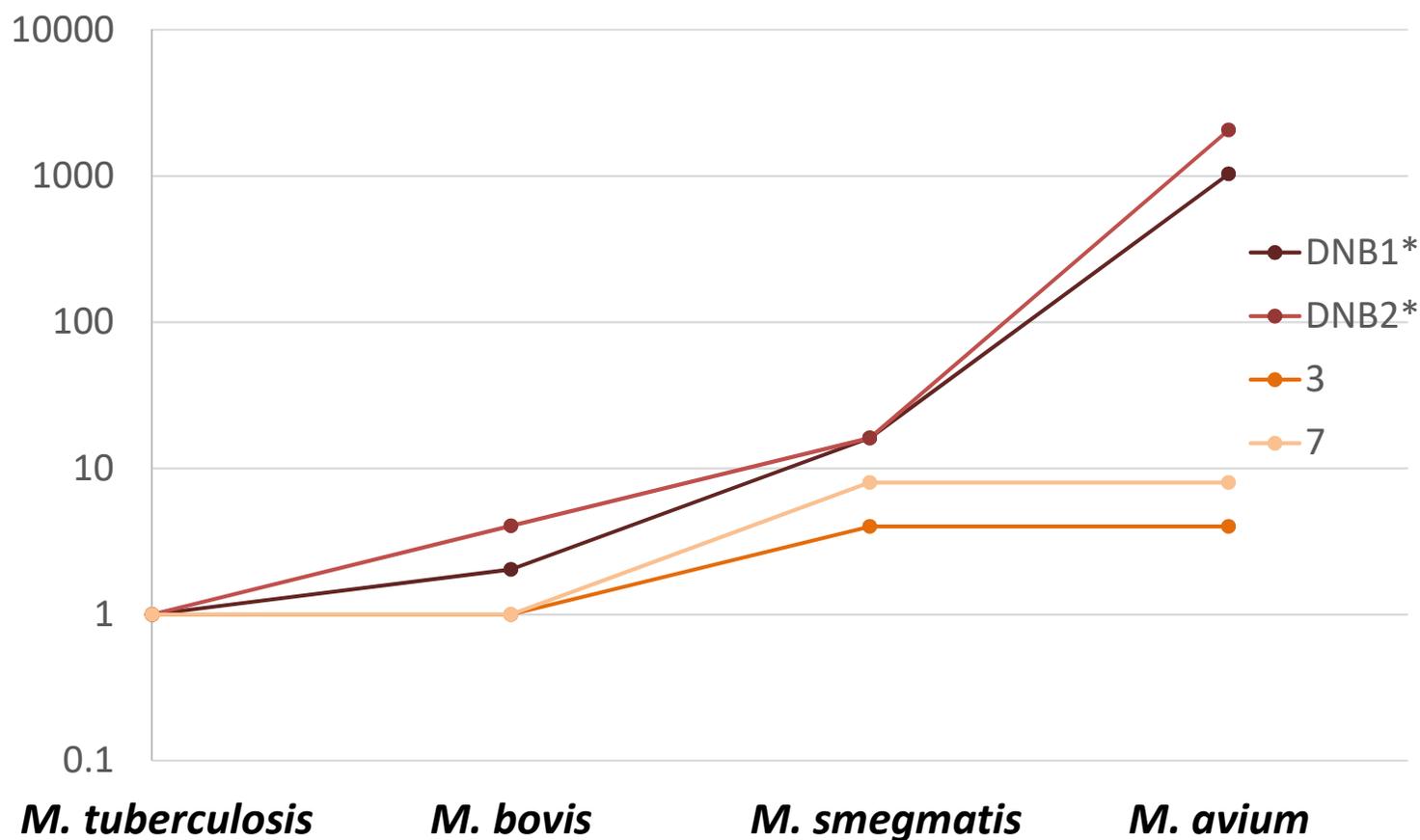
How much the MIC values increase in comparison to the values obtained for *M. tuberculosis*?





Results and Discussion: Multiple-strains assay

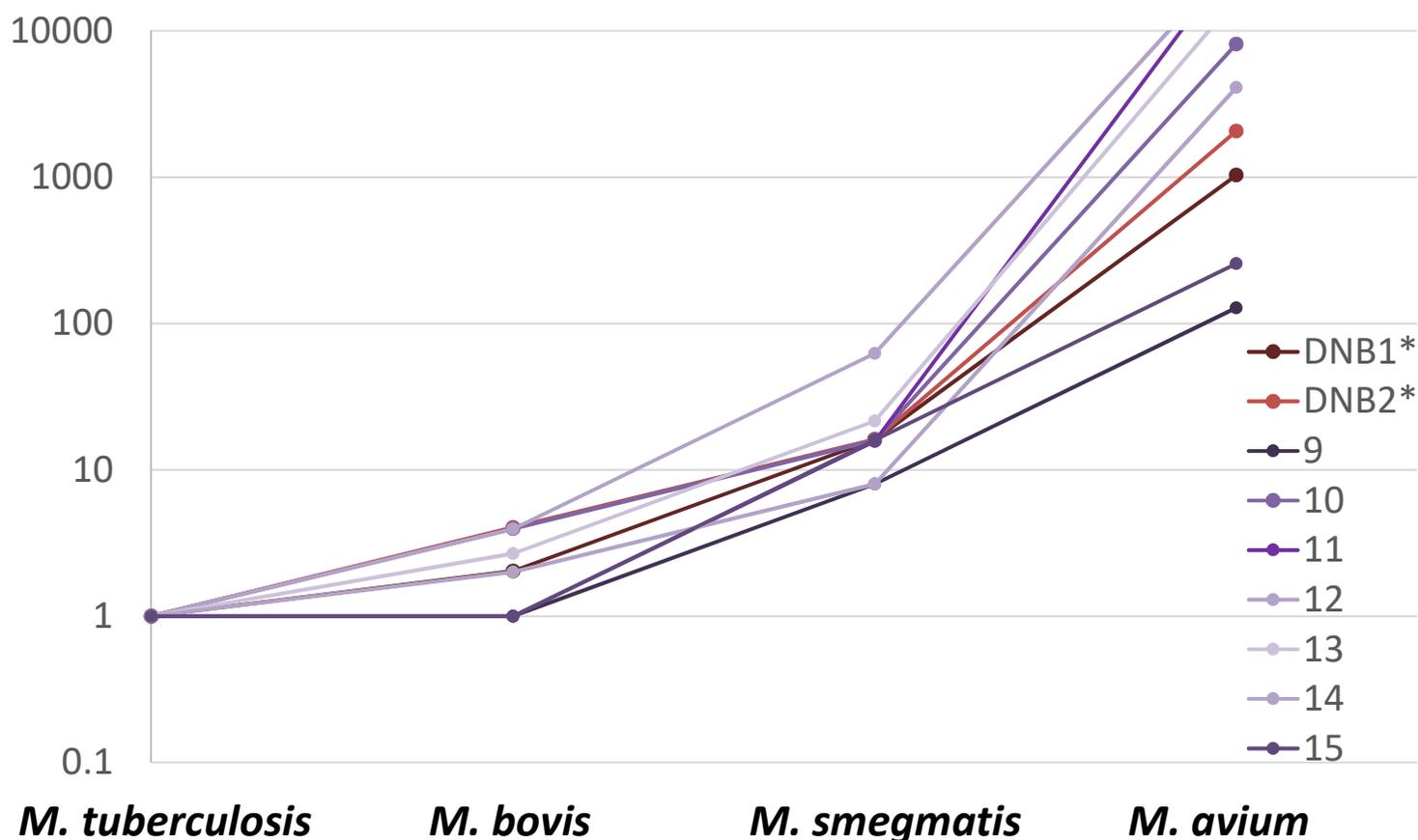
How much the MIC values increase in comparison to the values obtained for *M. tuberculosis*?





Results and Discussion: Multiple-strains assay

How much the MIC values increase in comparison to the values obtained for *M. tuberculosis*?





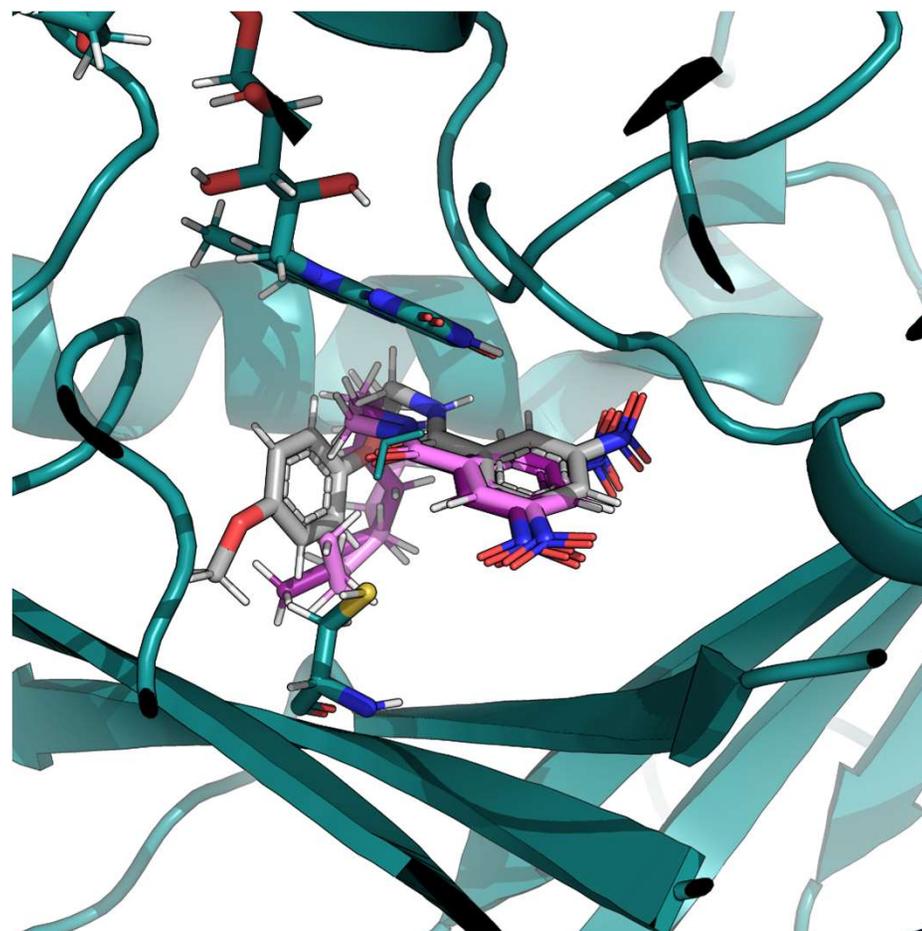
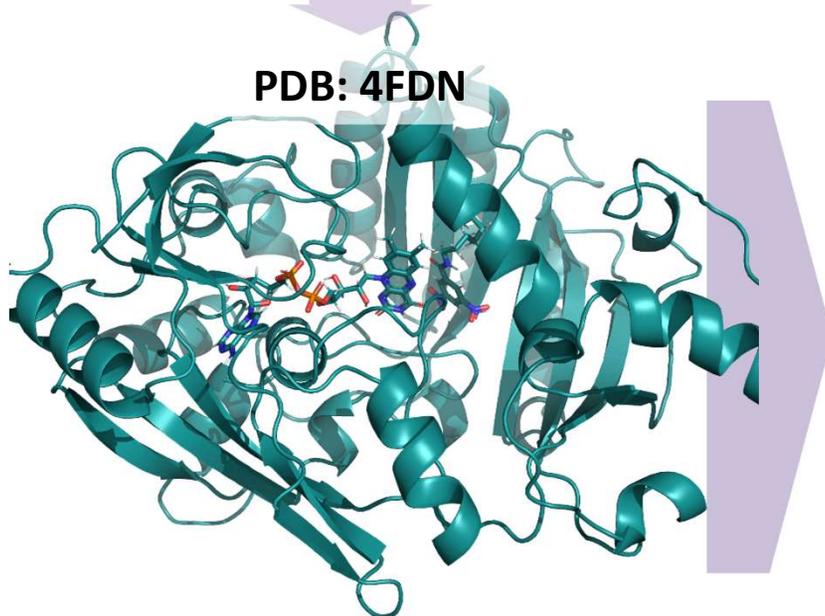
Results and Discussion: Computational studies

Docking approach

DprE1 crystallographic structures
(RCSB Protein Data Bank)

Selection by self-docking
and cross-docking

PDB: 4FDN





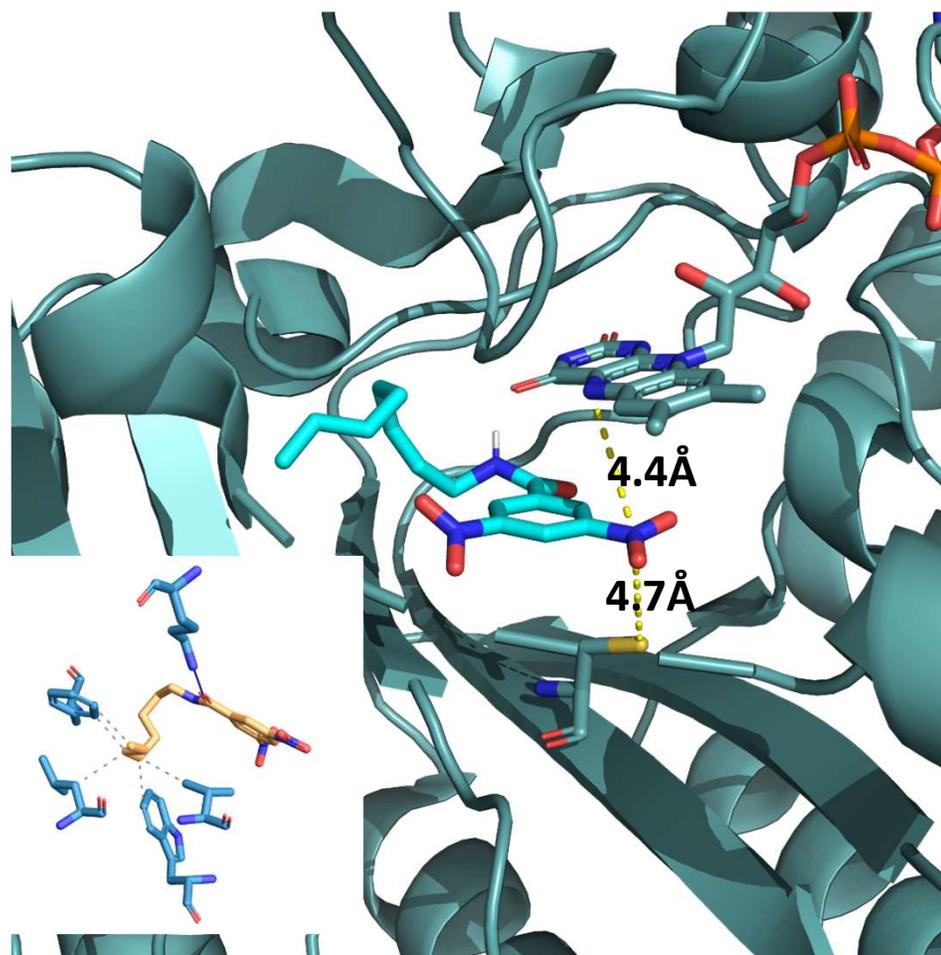
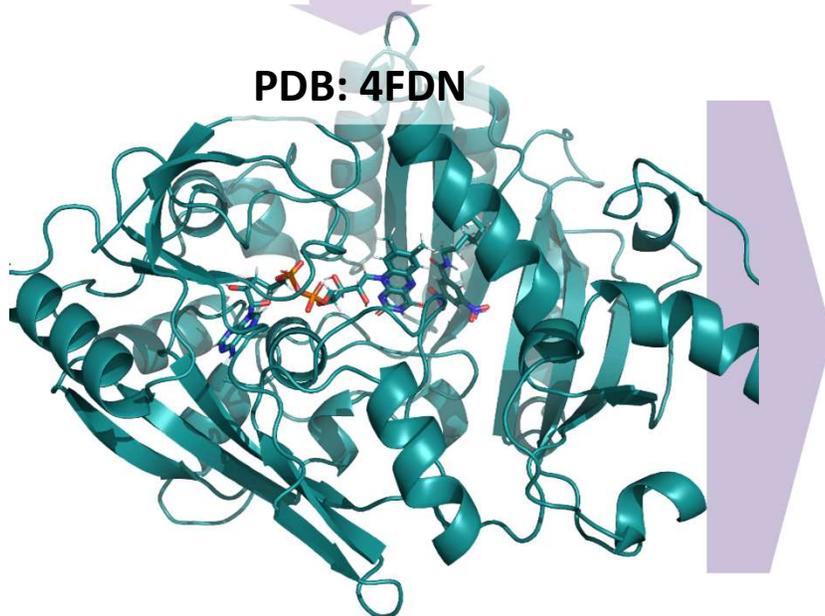
Results and Discussion: Computational studies

Docking approach

DprE1 crystallographic structures
(RCSB Protein Data Bank)

Selection by self-docking
and cross-docking

PDB: 4FDN





Conclusions

- **Synthesis and antitubercular activity of dinitrobenzamide alkyl derivatives** Accessible two-step synthesis with good yields

Optimal alkyl chain length range (8-10 carbon atoms)

Best-in-class antitubercular activities

- **Infection model**

Bacterial death induced with an efficacy comparable to INH

- **Mode of action**

Antibacterial activity profile to multiple strains similar to known DprE1 inhibitors

Docking studies show similar interaction profile to known DprE1 inhibitors

Distance of nitro-groups to FAD and Cys387 residue indicative of possible pre-reactive complex



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Acknowledgments

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