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Re-exploring the salicylanilide scaffold for the inhibition of mycolic acid synthesis in *Mycobacterium tuberculosis*

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Gwenaëlle Jézéquel, Said Aït Taleb, Chérine Mehalla, Nguyen-Hung Le, Messie Muipata-Muabilua, et al.. Re-exploring the salicylanilide scaffold for the inhibition of mycolic acid synthesis in *Mycobacterium tuberculosis*. Tackling antibacterial and antifungal resistant infections from disease to innovative therapies, Dec 2024, Paris, France. hal-04883625

HAL Id: hal-04883625

<https://hal.science/hal-04883625v1>

Submitted on 13 Jan 2025

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Re-exploring the salicylanilide scaffold for the inhibition of mycolic acid synthesis in *Mycobacterium tuberculosis*

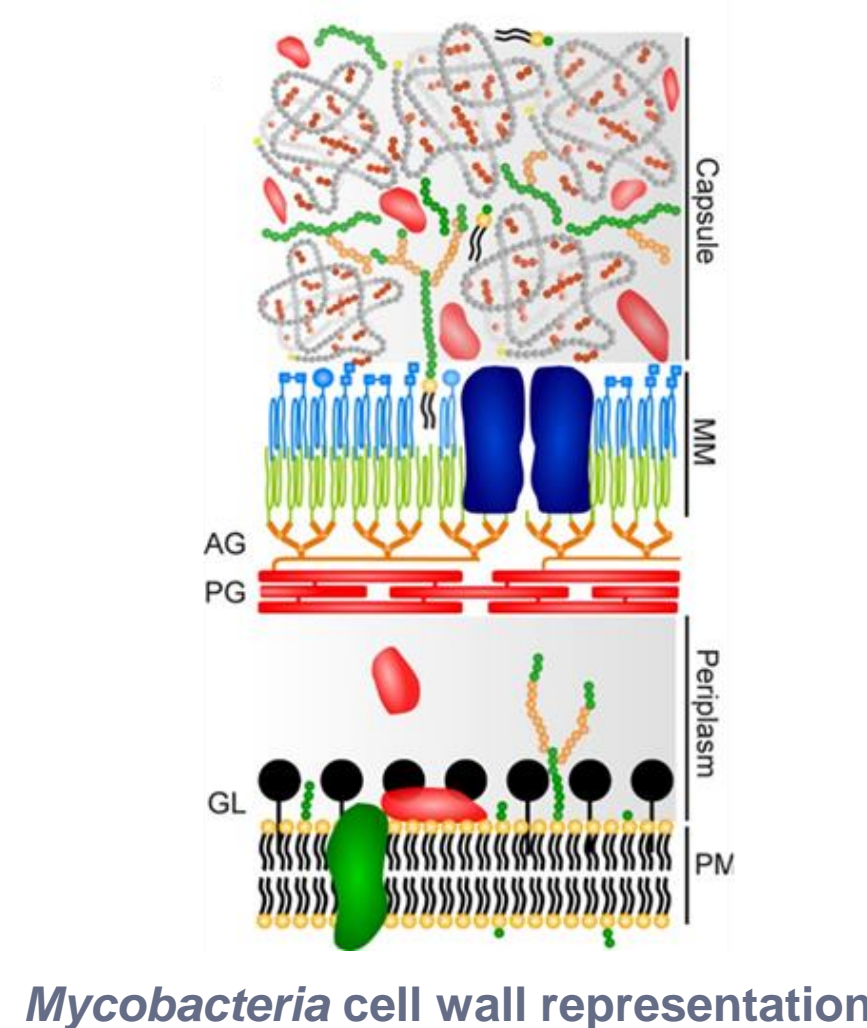
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Context and strategy

7.5 million of people diagnosed with tuberculosis in 2022

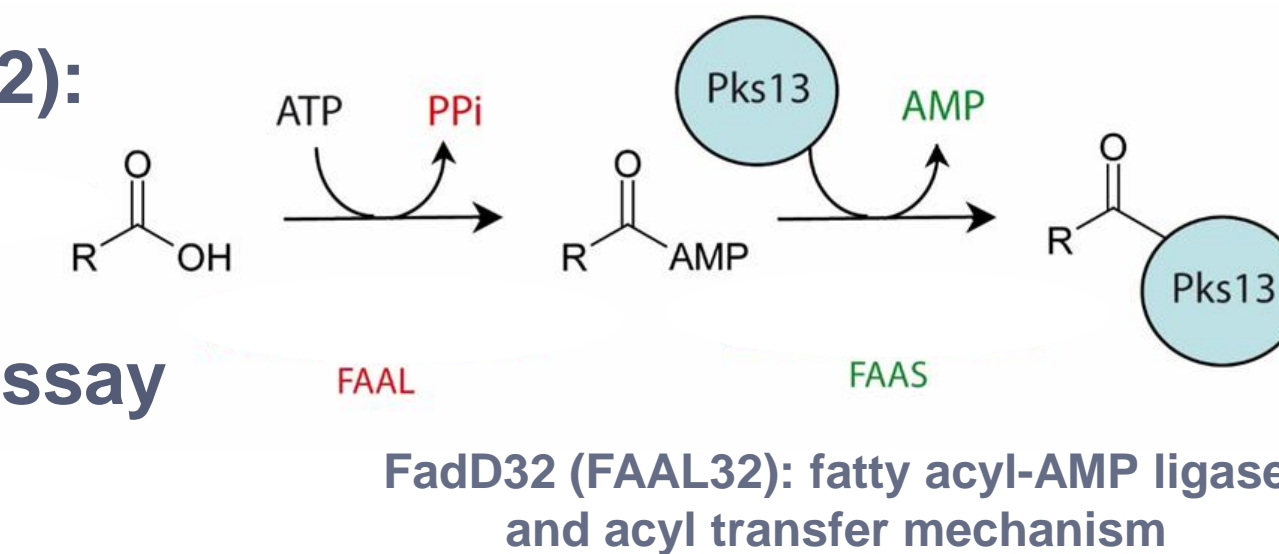
Mycolic acid (MA) synthesis:
 ✓ Essential for mycobacterial viability
 ✓ Validated drug target (isoniazid, delamanid)



Increase in *Mycobacterium tuberculosis* (Mtb) infections and antibiotic resistance

Urgent need to find new drugs with novel Mode of Action (MoA)

FadD32 acyl-AMP ligase (FAAL32):
 > Key-player in MA synthesis
 > Underexplored target
 > Newly developed enzymatic assay

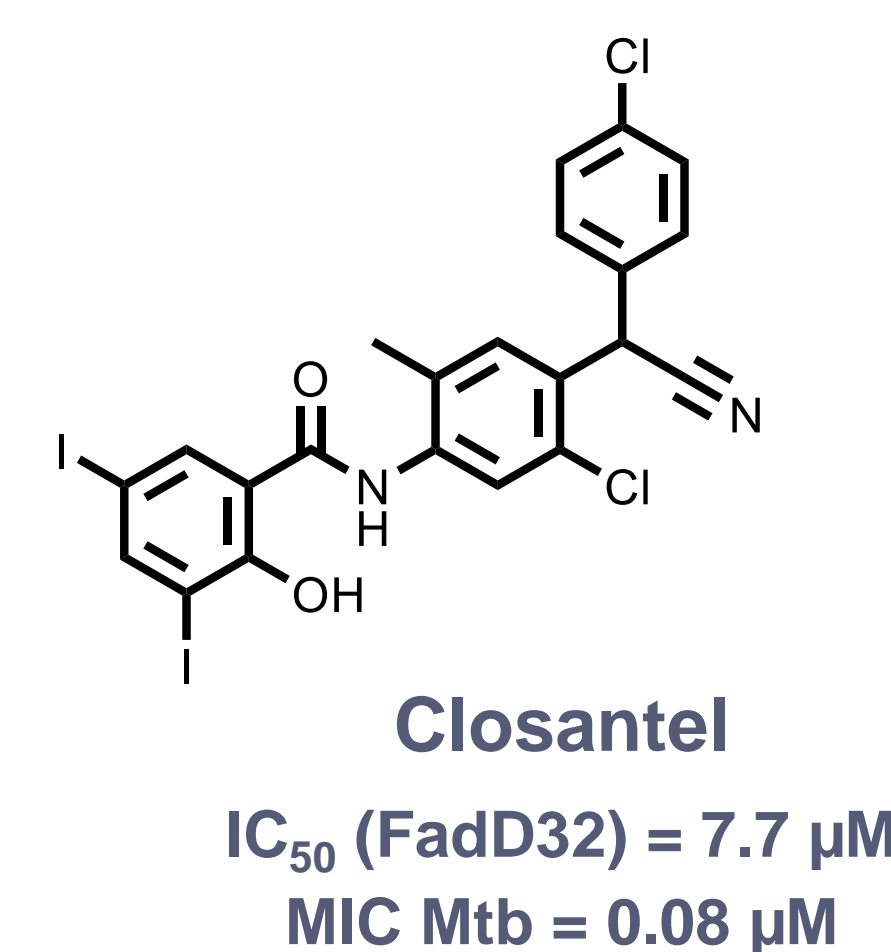
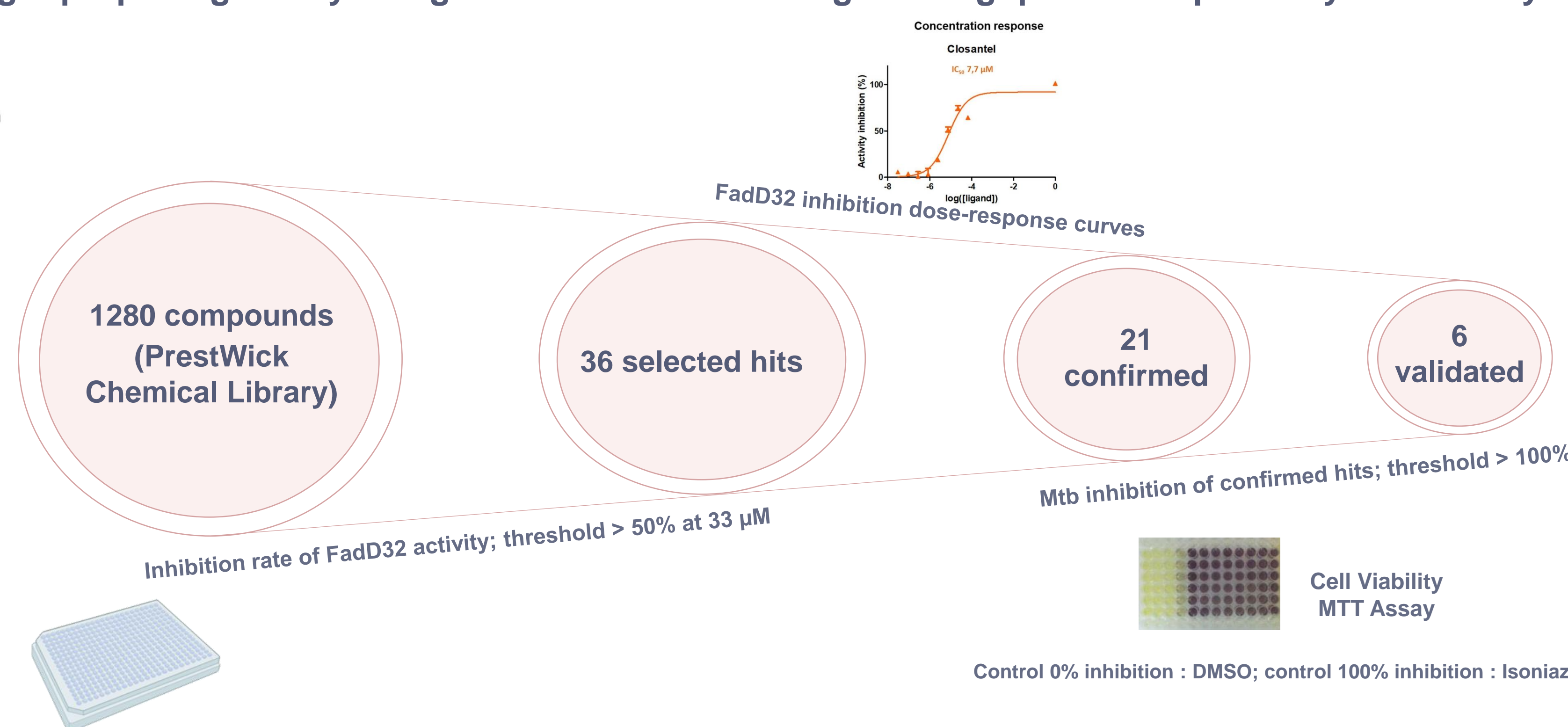
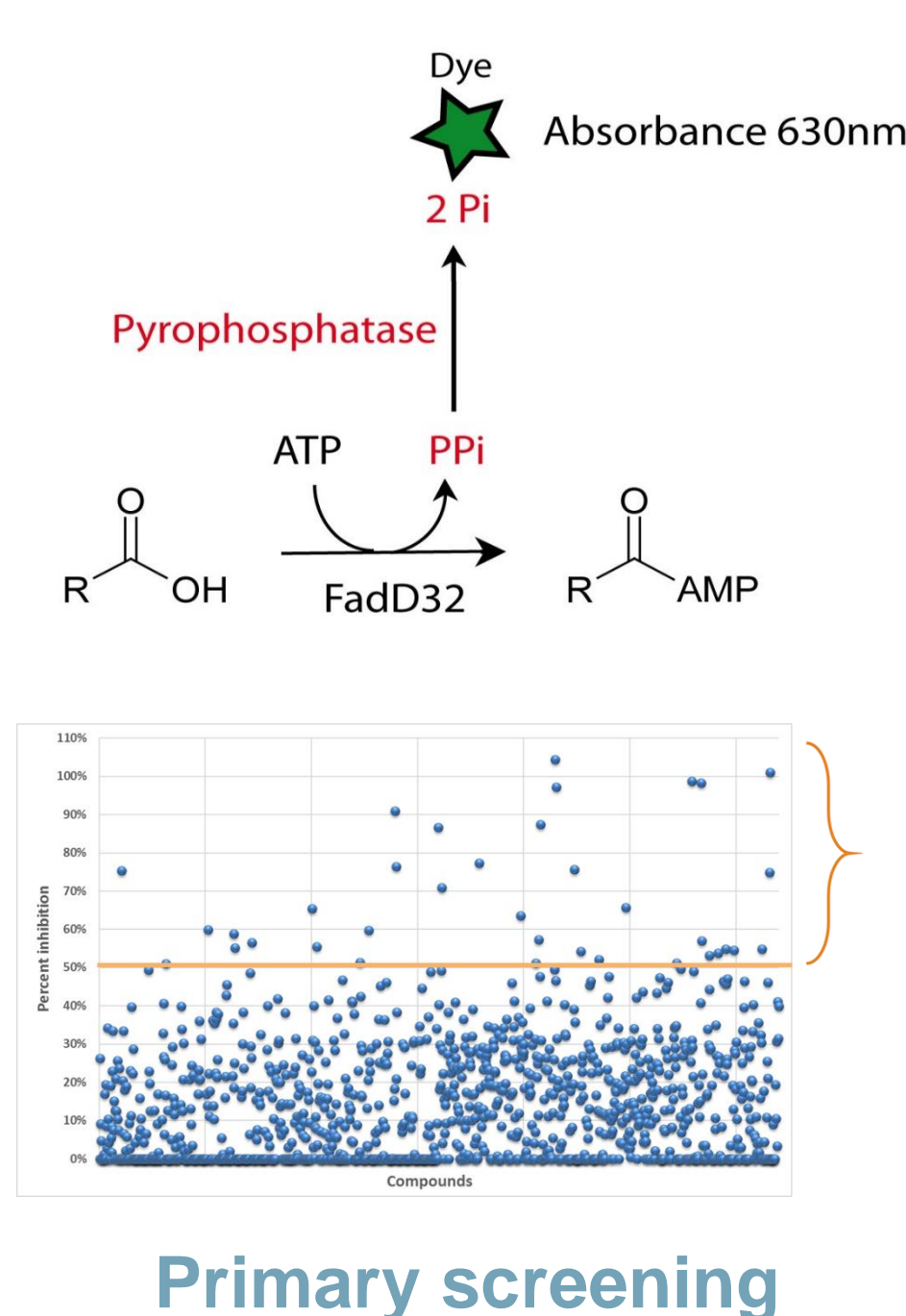


Our aim

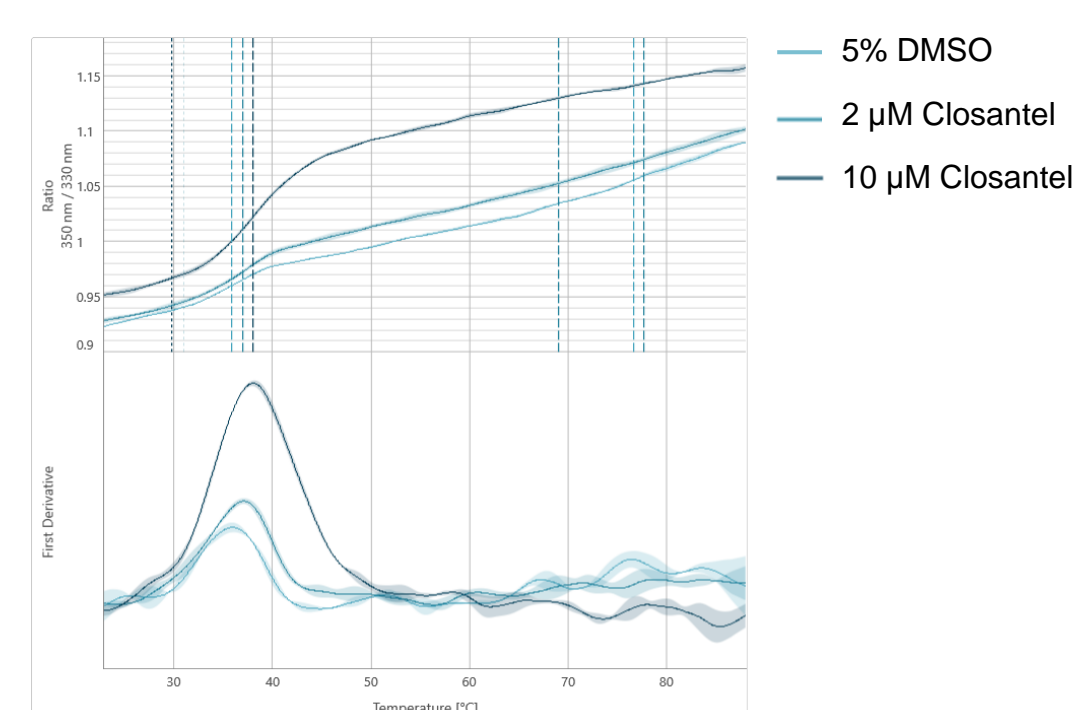
Identify new inhibitors of FadD32 to hamper MA synthesis and develop anti-tuberculosis agents

Results

Screening of a drug repurposing library using the semi-automated high-throughput developed enzymatic assay



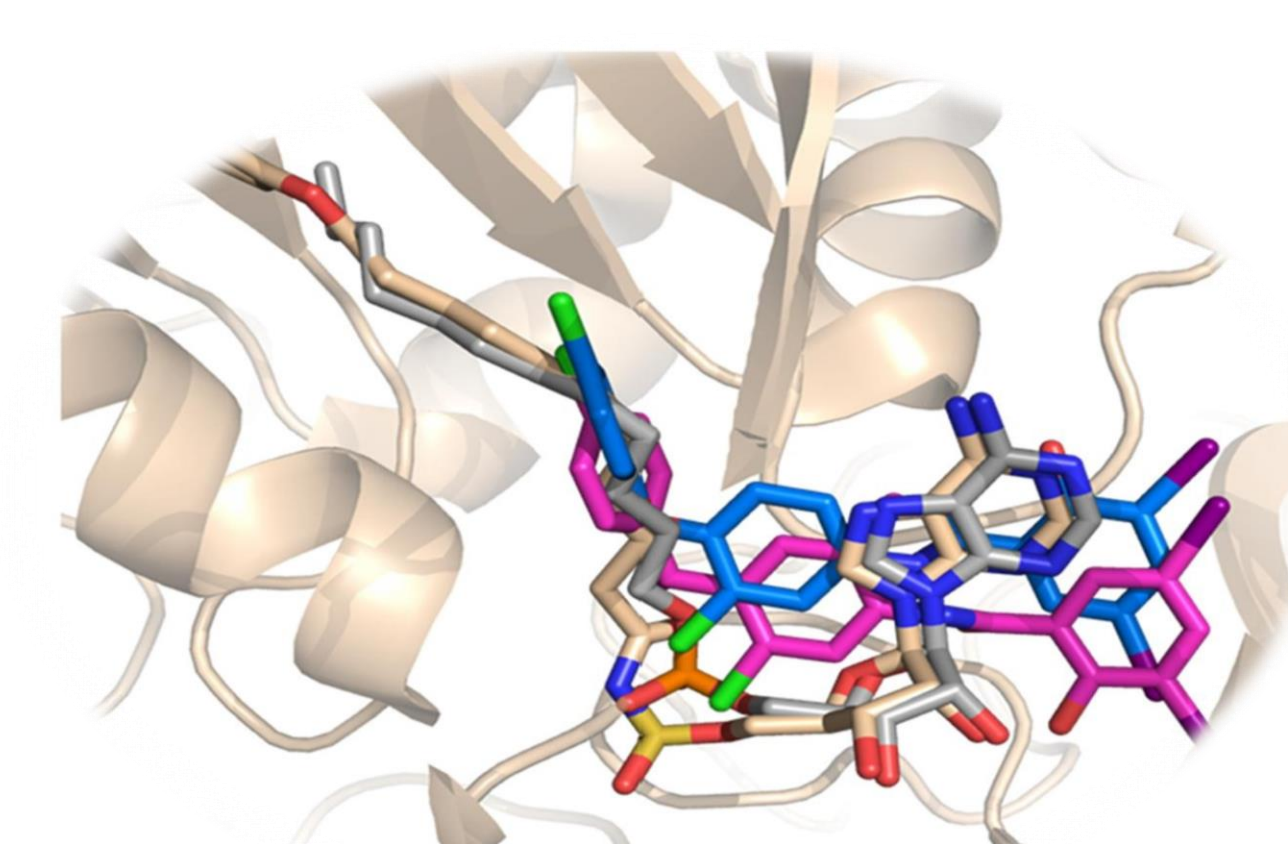
Measurement of protein (de)stabilization by nanoDSF
 Dose response answer of thermal unfolding
 ΔT_m (Closantel x5) = 2.1 °C



Assessment of FadD32 protein thermal unfolding in presence of closantel (FadD32 at 2 μM, Closantel at 0, 2 and 10 μM in 5% DMSO)

Binding affinity characterization

Molecular docking using AutoDock Vina

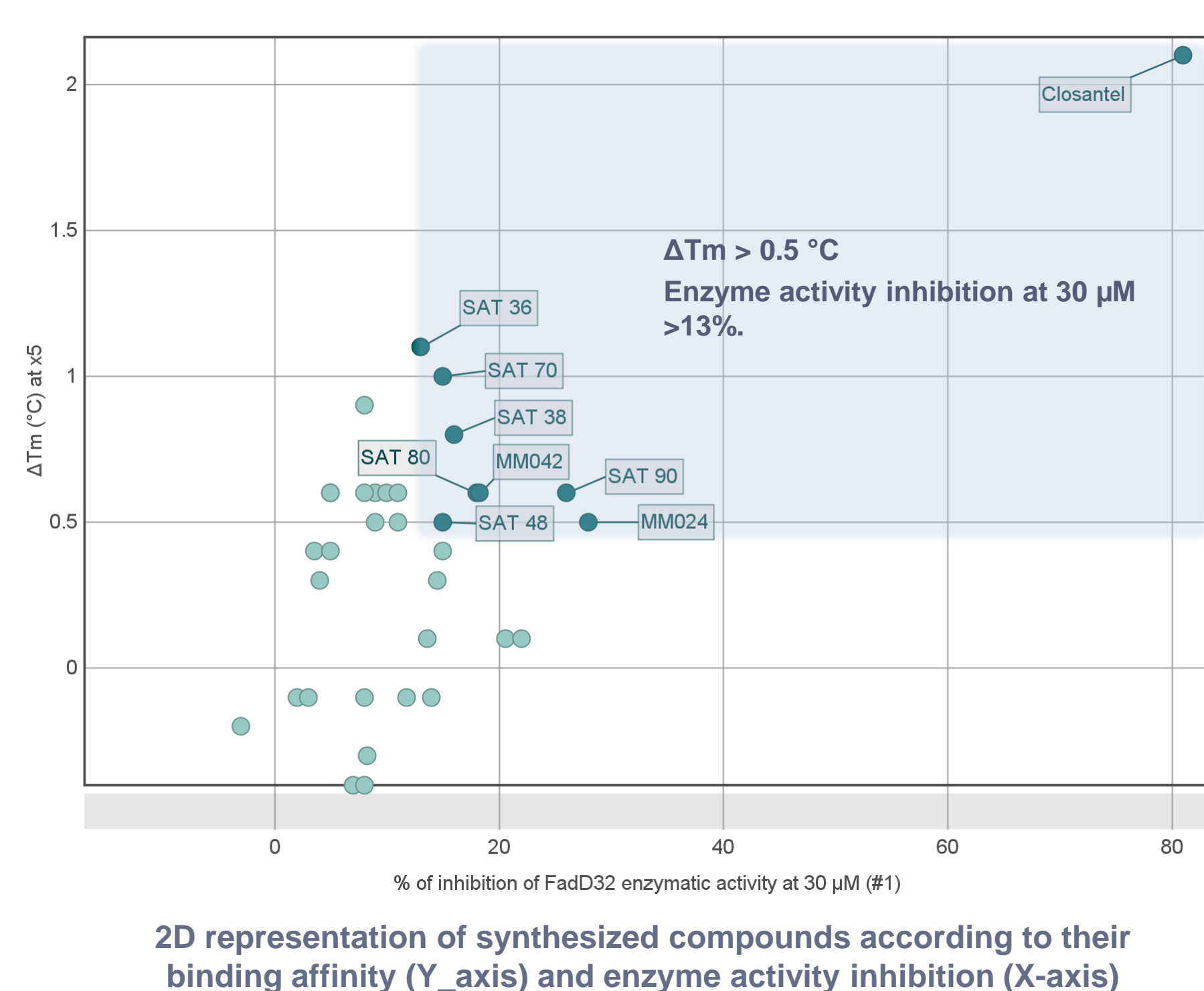


Le, N.-H. et al. *Bioorganic & Medicinal Chemistry* 71, 116938 (2022).

Superimposition of Mtb FadD32 in complex with AMPC12 molecule (gray) as observed in the structure of *M. marinum* FadD32 complex (PDB 5EY9), closantel (magenta) and rofoxamide (light blue), as docked into the Mtb FadD32 binding pocket

Medicinal chemistry optimisation

38 salicylanilide derivatives synthesized



Full biological characterization of compounds: binding affinity (nanoDSF), enzymatic activity and Mtb growth inhibition, cytotoxicity

Table summarizing data for the compounds presenting the highest Mtb growth inhibition

Compounds	Substituents	ΔT_m (°C) at x5	% of inhibition of FadD32 enzymatic activity at 30 μM	MIC on Mtb (μM)	CC ₅₀ on HepG2 (μM)
Closantel	/	2.1	81%	0.1	32.9
SAT 46	R ¹ = OH, R ² = R ³ = Cl	0.5	9%	10.0	13.6
SAT 52	R ³ = NO ₂ , R ⁵ = OH	0.1	22%	10.0	12.4
SAT 62	R ² = NO ₂ , R ⁵ = OH	0.6	10%	5.0	17.6
SAT 66	R ² = I, R ³ = OH	0.6	5%	1.3	14.5
SAT 70	R ¹ = OH, R ² = R ⁴ = Br	1.0	15%	2.5	26.6
SAT 80	R ¹ = OH, R ² = R ⁴ = Cl	0.6	18%	10.0	30.0
SAT 82	R ¹ = OH, R ⁴ = Cl	-0.4	8%	5.0	14.4
SAT 84	R ¹ = OH, Z = N	0.6	8%	2.5	32.4
SAT 90	R ¹ = OH, R ² = R ⁴ = I	0.6	26%	1.3	32.5

Conclusion

A new salicylanilide pharmacophore was identified after an extensive screening as a promising FadD32 inhibitor towards anti-tuberculosis drug development

This pharmacophore has been derivatized and evaluated (affinity, enzymatic and phenotypic inhibition) to determine SAR of this class

Phenol in ortho position, as well as (di)-halogenation in ortho/meta-positions seem beneficial for the activity of this class on FadD32 and for phenotypic activity

Outlook

Further derivatization to obtain a set of potential lead compounds

PK/PD studies

Validation of mode of action (inhibition of FadD32 and of mycolic acid synthesis)