Screening for novel inhibitors of *Mycobacterium tuberculosis* dihydrofolate reductase using structure-based virtual screening

Elena Cubero [1], Simon Bray [1], Rinaldo W. Montalvão [1], Maria Aiza Fontes Andrade [2], Ana María García-Collazo [1], and Marcio Vinícius Bertacine Dias [2]

[1] Gain Therapeutics, Barcelona, Spain and Bethesda, MD, USA; [2] Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

ecubero@gaintherapeutics.com

Magellan™ transforms ultra-large, synthetically accessible chemical space into experimentally validated starting points for *Mtb*DHFR inhibitor discovery

Therapeutic challenge

Tuberculosis remains a major global health challenge, exacerbated by the presence of multidrug-resistant strains of *Mycobacterium tuberculosis*.

*Mtb*DHFR is essential enzyme for folate metabolism and an attractive target for the development of new anti-tuberculosis agents.

Structural starting point

*Mtb*DHFR crystal structure (PDB ID: 4KL9)[1] was used to assess active-site key interaction hotspots.

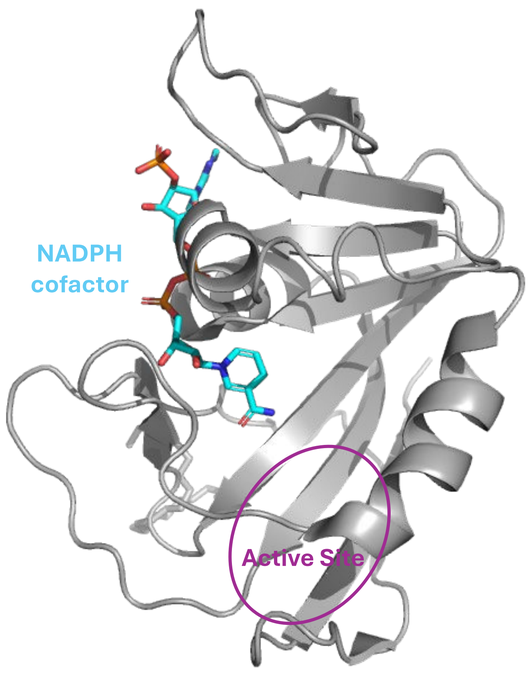


Figure 1. Crystal structure of *M. tuberculosis* dihydrofolate reductase (*Mtb*DHFR; PDB ID: 4KL9), shown as a grey cartoon with the NADPH cofactor represented as sticks. The purple ellipse highlights the established catalytic active site.

Hotspot-guided screening hypothesis

Mixed-solvent molecular dynamics (MDmix) identified interaction hotspots that defined pharmacophoric requirements for virtual screening.

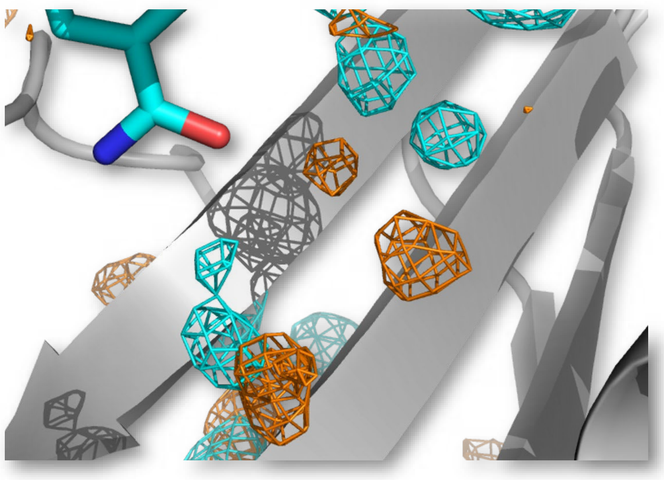
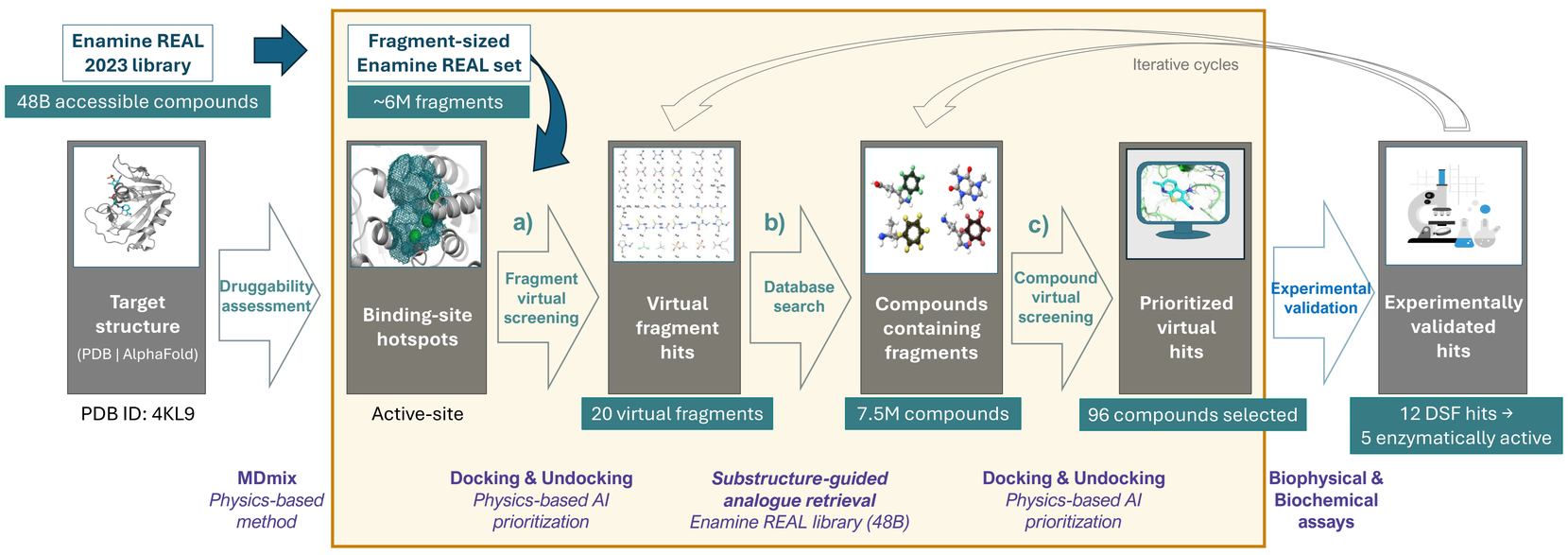


Figure 2. Contour plots showing the optimal interaction sites of a hydrophobic probe (methyl of ethanol; cyan mesh) and a polar probe (hydroxyl of ethanol; orange mesh), both at ΔG_{bind} = -0.5 kcal/mol.

[1] Dias et al. (2014) *Structure* 22, 94–103

Magellan™ workflow

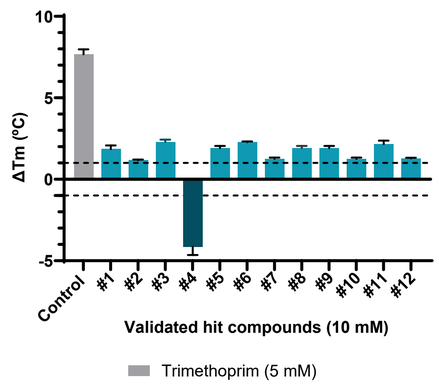
1. **Target structure selection.** A high-quality target protein structure is selected from the Protein Data Bank (PDB) or generated using AlphaFold
2. **Binding-site and druggability assessment.** MDMix simulations identify key interaction hotspots within the target binding site and define the pharmacophoric requirements for screening
3. **Fragment-space definition.** A synthetically accessible, fragment-sized subset is extracted from the Enamine REAL chemical space
4. **AI-enabled fragment prioritization.** Proprietary data-trained docking and DUck models on prioritize fragments that satisfy the predefined structural and interaction criteria
5. **Fragment expansion.** The highest-priority fragments are used as substructures to retrieve larger, synthetically accessible analogues from the Enamine REAL library
6. **Molecule-level prioritization.** The expanded compound set is ranked using docking and DUck-based evaluation to select on-demand compounds for experimental testing
7. **Orthogonal experimental validation.** Selected compounds are tested using orthogonal DSF, enzymatic inhibition and ITC assays



Experimental validation at University of São Paulo

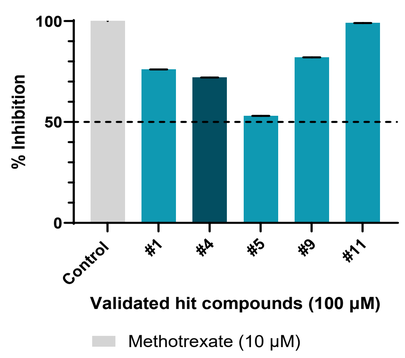
Sequential experimental validation with recombinant *Mtb*DHFR: DSF → enzyme inhibition → ITC

a) DSF
Target engagement



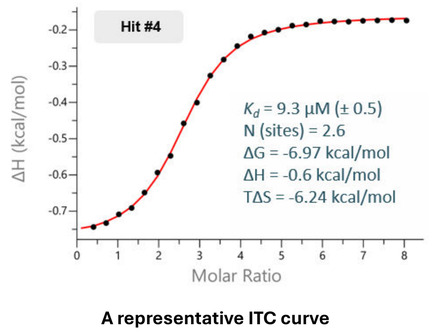
12 hits; ΔT_m: −4.5 to +2.38°C

b) Enzyme inhibition
Functional activity



5 compounds with ≥50% inhibition

c) ITC
Binding affinity



A representative ITC curve

5 inhibitors advanced; 4 yielded K_d values of 8.96 µM–1.65 mM

Follow-up exploration: Selectivity profiling is ongoing

Conclusions

- Active-site hotspot analysis defined the pharmacophore and guided the Magellan™ screening strategy
- A hierarchical, AI-enabled workflow narrowed a 48-billion-compound, synthetically accessible chemical space to a focused set of candidates for experimental testing
- Orthogonal biophysical and biochemical assays identified new chemical starting points that bind *Mtb*DHFR and inhibit its enzymatic activity

DEPARTAMENTO DE MICROBIOLOGIA UNIVERSIDADE DE SÃO PAULO · ICB USP · USP

GAIN THERAPEUTICS www.gaintherapeutics.com

SPANISH Drug Discovery Network www.sddn.es/programme2026/