Predictive power of in silico approach to evaluate chemicals against M. tuberculosis: A systematic review

Giulia Timo¹, Rodrigo dos Reis¹, Adriana de Melo¹, Thales Costa¹, Pérola Magalhães², Mauricio Homem-de-Mello¹*

¹ InSiliTox, Department of Pharmacy, Faculty of Health Sciences, University of Brasilia, Brasilia 70910-900, Brazil;
² Laboratory of Natural Products, Department of Pharmacy, Faculty of Health Sciences, University of Brasilia, Brasilia 70910-900, Brazil.

* Corresponding author: mauriciohmello@unb.br
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Graphical Abstract

Research on databases:
- PUBMED (n = 632)
- WEB OF SCIENCE (n = 929)
- SCIENCE DIRECT (n = 863)

TOTAL: 2424 manuscripts

Screening using EndNote™

FINAL: 46 suitable manuscripts

Additional studies from references:
- 6 manuscripts

Abstract and full paper reading: 72 manuscripts
Abstract: Tuberculosis is still one of the most prevalent diseases worldwide caused by *Mycobacterium tuberculosis* (Mtb), bearing a long-term treatment that is not always effective. Admitting this context, multiple studies have been trying to develop novel substances against Mtb, specially using *in silico* techniques to predict its effects on a known target. Using a systematic approach, we were able to retrieve and evaluate 46 manuscripts from three different databases that firstly applied an *in silico* technique to explore new antimycobacterial molecules and secondly attempted to prove its predictive potential by an *in vitro* or *in vivo* assay. We found that although all manuscripts followed a similar screening procedure (ligand and/or structure-based screening), they explored a large number of ligands on 29 distinct bacterial enzymes. The following *in vitro/vivo* analysis showed that the virtual screening was able to decrease the number of tested molecules, saving time and funding, but could only provide a modest correlation to the effectiveness of those molecules *in vitro*. In short, we found that the preliminary *in silico* approach is recommended specially on the early steps in developing a new drug, but call for more studies to evaluate its clinical predictive possibilities.

Keywords: *Mycobacterium tuberculosis*; tuberculosis; *in silico*; virtual screening; docking.
Introduction

- According to the latest World Health Organization (WHO) report, tuberculosis (TB) is still one of the top 10 causes of death and the leading cause from a single infectious agent (even above HIV/AIDS) [1].

- Also, multidrug-resistant TB (MDR/TB) and extensively drug-resistant TB (XDR/TB) have been increasing over the years, resulting in loss of effect of first and second lines of anti-TB drugs, like Rifampicin and Isoniazid [2].

[1] WHO. Global Tuberculosis Report; WHO: Geneva, Switzerland, 2018; p. 277.
[2] Gandhi, N.R, et al. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. Lancet 2010, 375, 1830–1843.
Introduction

• In silico drug screening can be divided into two main paths:

1. **Ligand-based drug screening** → uses data available about inhibitors that will be studied in several methods (such as quantitative structure-activity relationship or QSAR) [3].

2. **Structure-based drug screening (SBDS)** → uses data available about 3D shapes of targets that will be inhibited, using a docking program (such as GLIDE) to screen a large database of compounds (such as ZINC) to identify hit molecules through docking score analysis [4].

[3] Mehra, R., et al. Discovery of new Mycobacterium tuberculosis proteasome inhibitors using a knowledge-based computational screening approach. Mol. Divers. 2015, 19, 1003–1019.
[4] Lengauer, T.; Rarey, M. Computational methods for biomolecular docking. Curr. Opin. Struct. Biol. 1996, 6, 402–406.
Introduction

• To further refine the obtained in silico results, it is often necessary for researchers to perform an in vitro or in vivo assay to confirm their virtual hit results [5,6,7].

• Based on this background, this study aimed to collect all the research published until 15 August 2018 that performed at least one of the in silico methods cited previously and corroborated the results with an in vitro or in vivo assay, succeeding at a critical analysis of the obtained results.

[5] Saxena, S., et al. Identification of novel inhibitors against Mycobacterium tuberculosis L-alanine dehydrogenase (MTB-AlaDH) through structure-based virtual screening. J. Mol. Graph. Model. 2014, 47, 37–43.
[6] Cinu, T.A., et al. Design, synthesis and evaluation of antitubercular activity of Triclosan analogues. Arab. J. Chem. 2015.
[7] Samala, G., et al. Identification and development of 2-methylimidazo[1,2-a]pyridine-3-carboxamides as Mycobacterium tuberculosis pantothenate synthetase inhibitors. Bioorganic Med. Chem. 2014, 22, 4223–4232.
Materials and Methods

Identification
- PubMed (n = 632)
- Web of Science (n = 929)
- Science Direct (n = 863)

Records identified through database searching (n = 2424)

Records after duplicate / triplicate removal (n = 1645)

Screening
- First filter (Mycobacterium tuberculosis in title or abstract) (n = 1149)
- Second filter (docking or docked or QSAR or virtual screening in abstract) (n = 836)
- Third filter (in vitro or in vivo or IC50 or EC50 or MIC in abstract) (n = 237)

Eligibility
- Individual critical reading
  - Exclusion criteria
    - Fitting outside the review scope
    - Just citing the terms in the text (n = 72)
- Group discussion
  - Harmonization of exclusion and inclusion criteria (n = 40)

Inclusion
- Additional studies included during data collection and review process (n = 6)

Selected Studies (n = 46)
Results and discussion

1. *Mycobacterium tuberculosis* Enzyme Targets

- Enoyl-[acyl-carrierprotein] reductase (NADH) 55%
- DNA topoisomerase (ATP-hydrolyzing) 20%
- DNA topoisomerase I 9%
- DNA ligase (NAD (+)) 7%
- Shikimate kinase 4%
- Other enzymes (one each) 5%

Additional information and references are listed in Timo, G.O, et al. Predictive Power of In Silico Approach to Evaluate Chemicals against *M. tuberculosis*: A Systematic Review. *Pharmaceuticals* 2019, 12, 135. DOI: [https://doi.org/10.3390/ph12030135](https://doi.org/10.3390/ph12030135)
Results and discussion

1. *Mycobacterium tuberculosis* Enzyme Targets

- We found 29 distinct targets within 46 papers with different effects on bacterium survival.
- The most exploited Mtb enzyme was Enoyl-[acyl-carrier-protein] reductase (NADH) (EC 1.3.1.9), studied 9 times.
- This shows that despite increasing evidence of Mtb resistance, there are still many efforts in the search for novel targets.
- However, few drugs are actually being released into the pharmaceutical market.
Results and discussion

2. PDB

Additional information and references are listed in Timo, G.O, et al. Predictive Power of In Silico Approach to Evaluate Chemicals against *M. tuberculosis*: A Systematic Review. *Pharmaceuticals* 2019, 12, 135. DOI: [https://doi.org/10.3390/ph12030135](https://doi.org/10.3390/ph12030135)
Results and discussion
2. PDB

• We found 40 different PDBs analyzed within the 46 retrieved manuscripts.

• The use of PDBs was seen for both structure- and ligand-based screening.
• This finding means that the crystal structures of a determined protein can be used to study the interaction between atoms of a targeted structure and a postulated inhibitor, thereby useful to develop novel scaffolds for lead optimization.
Results and discussion

3. Virtual Screening Methods Applied

- After the evaluation of all 46 documents, we found that there was a balance between the presence of both methods.

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Results and discussion
4. Databases Screened

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Results and discussion

5. Docking Software Employed

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Results and discussion
6. In Vitro or In Vivo Testing

- Cytotoxicity Assay: 27%
- Enzymatic Inhibition (IC50): 33%
- Minimum Inhibitory Concentration (MIC): 40%

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Results and discussion
6. *In Vitro* or *In Vivo* Testing

- After collecting all displayed data, we analyzed whether the *in silico* methodologies were accurate for predicting the best possible MIC (which would be the **lowest** value).
- For this analysis, we searched if authors performed a control with a standard anti-TB drug (such as isoniazid, rifampicin, etc.).
- If there was not a control available, we developed our own method to evaluate if their new compound was effective:

  - Summation of all MIC values from most potent drug (**Isoniazid**)
  - Performed a mean MIC value (excluding outliers with z-score higher than 3)
  - $\text{MIC} = 0.78 \, \mu\text{M}$
Results and discussion

6. In Vitro or In Vivo Testing

• Applying the MIC value obtained from Isoniazid (0.78 µM) and the ones presented by each respective author as control, we also performed a ratio value to analyze if the MICs for their new compounds were more or less effective than approved drugs.

\[
\frac{\text{MIC from new developed molecule}}{\text{MIC from standard approved drug}}
\]

• Molecules were considered excellent if they had MIC ratio below or close to 1 \(\rightarrow\) meaning that new compound was more effective or equally effective to the control.
Results and discussion

6. *In Vitro* or *In Vivo* Testing

- From all manuscripts that performed the MIC assay (30), we found only 11 documents that presented excellent MIC, superior to at least one of the tested/calculated controls.

- All other documents had average MIC, not superior to the tested controls.

Extra information about compound nomenclatures and molecular structures, MICs, ratio values, IC$_{50}$, and docking scores are shown in Table 2 from Timo, G.O, et al. Predictive Power of In Silico Approach to Evaluate Chemicals against *M. tuberculosis*: A Systematic Review. *Pharmaceuticals* 2019, 12, 135. DOI: [https://doi.org/10.3390/ph12030135](https://doi.org/10.3390/ph12030135).
Results and discussion

6. In Vitro or In Vivo Testing

- Molecular structures from compounds with best MIC ratio

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Results and discussion
6. In Vitro or In Vivo Testing
• Molecular structures from compounds with best MIC ratio

Extra information about compound nomenclatures and molecular structures, MICs, ratio values, IC$_{50}$, and docking scores are shown in Table 2 from Timo, G.O, et al. Predictive Power of In Silico Approach to Evaluate Chemicals against *M. tuberculosis*: A Systematic Review. *Pharmaceuticals* 2019, 12, 135. DOI: [https://doi.org/10.3390/ph12030135](https://doi.org/10.3390/ph12030135).
Results and discussion

7. Validation procedures

• Validation of virtual screening procedures or in silico methodologies are not mandatory, but it is often seen.

Did not perform validation assay 59%

Performed validation assay 41%

• Validations experiments considered:
  1. Redocking the targeted protein and its original ligand;
  2. Comparison between binding conformations of the found molecules and the original ligands on the targeted protein;
  3. Molecular dynamics simulations.
Results and discussion

8. Timeline Analysis of Retrieved Manuscripts

- As it was our aim to present a wide view under this theme, we researched all manuscripts published until date 15 August that met our eligibility criteria.
- After thorough exclusion, we were able to collect 46 documents, ranging from 2005 to 2018.
Conclusions

1) Preliminary virtual screening methods were indeed able to aid researchers rank best scoring compounds, saving time and funding.
2) However, only a few scaffolds obtained from in silico studies maintained in vitro activities and are suitable for further assays.
3) It was seen that this outcome was obtained regardless of virtual methods, databases, docking softwares and validation procedures.
4) This study means that in silico methodologies needs to be further explored to yield better outcomes, but its use is still recommended, specially on the early steps in developing a new pharmaceutical drug against Tuberculosis.
Acknowledgments

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