5th International Electronic Conference on Medicinal Chemistry
1-30 November 2019
chaired by Dr. Jean Jacques Vanden Eynde
sponsored by pharmaceuticals

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

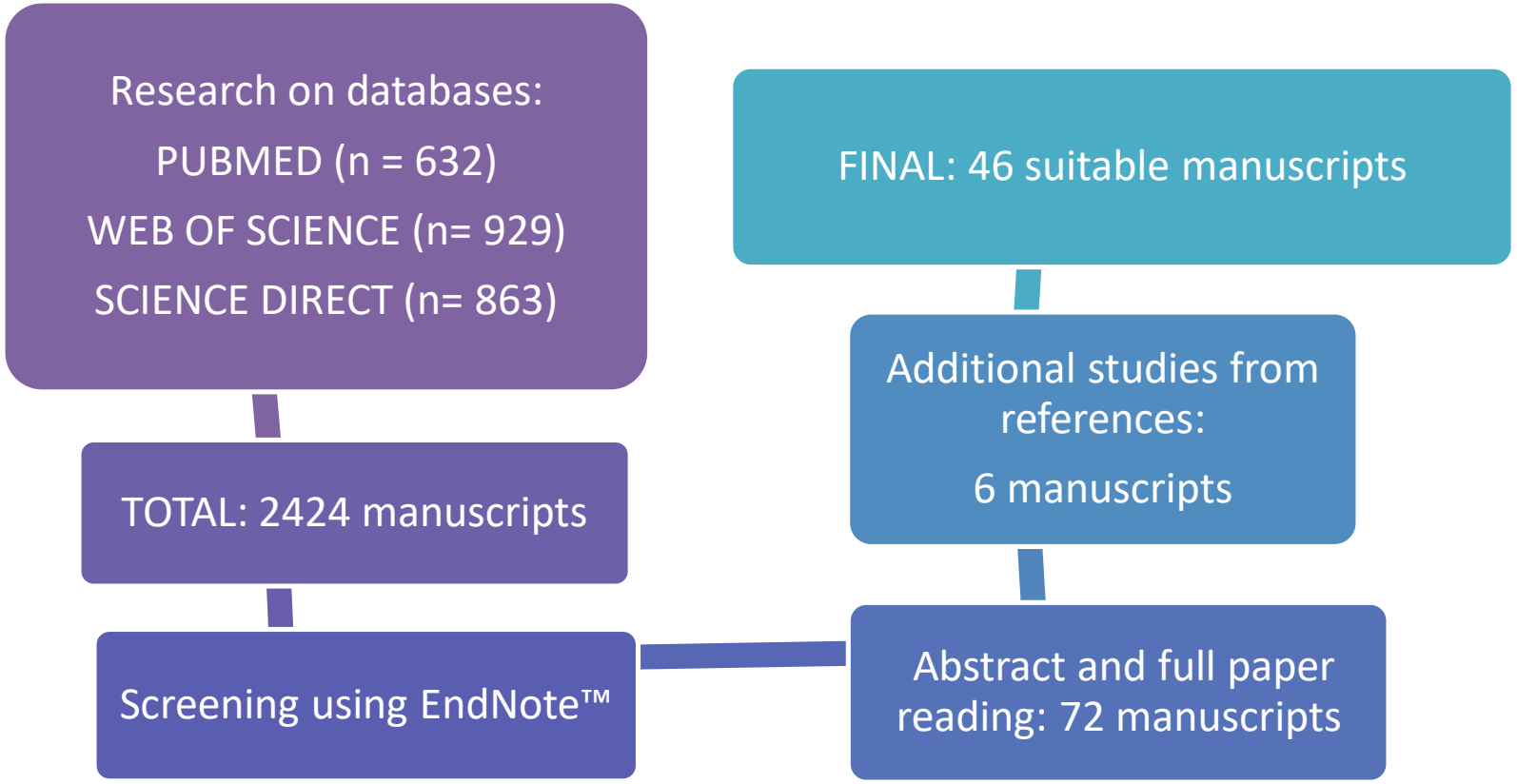
Giulia Timo[1], Rodrigo dos Reis[1], Adriana de Melo[1], Thales Costa[1], Pérola Magalhães[2], Mauricio Homem-de-Mello[1]*

[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

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

Graphical Abstract



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.

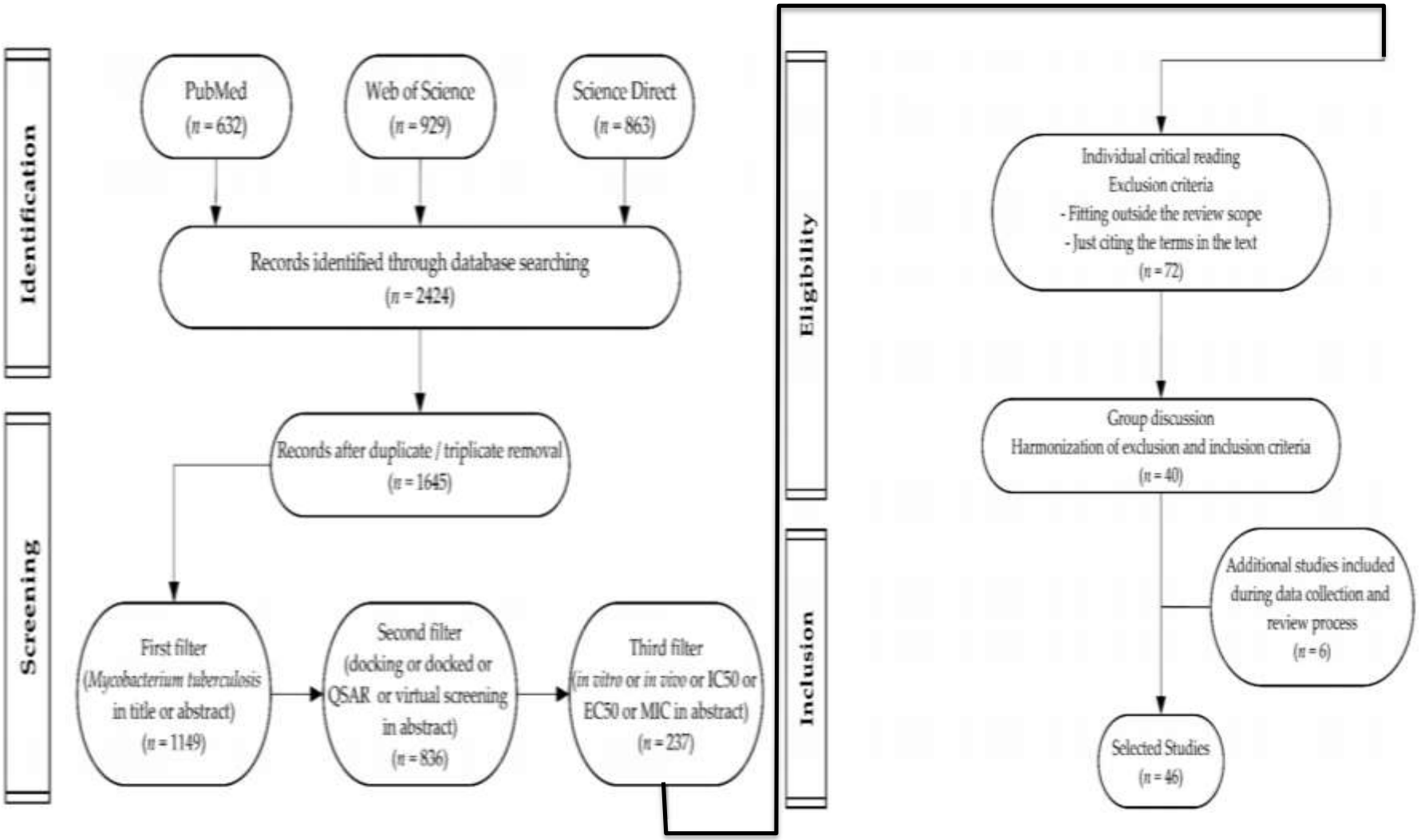
Introduction

- 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.

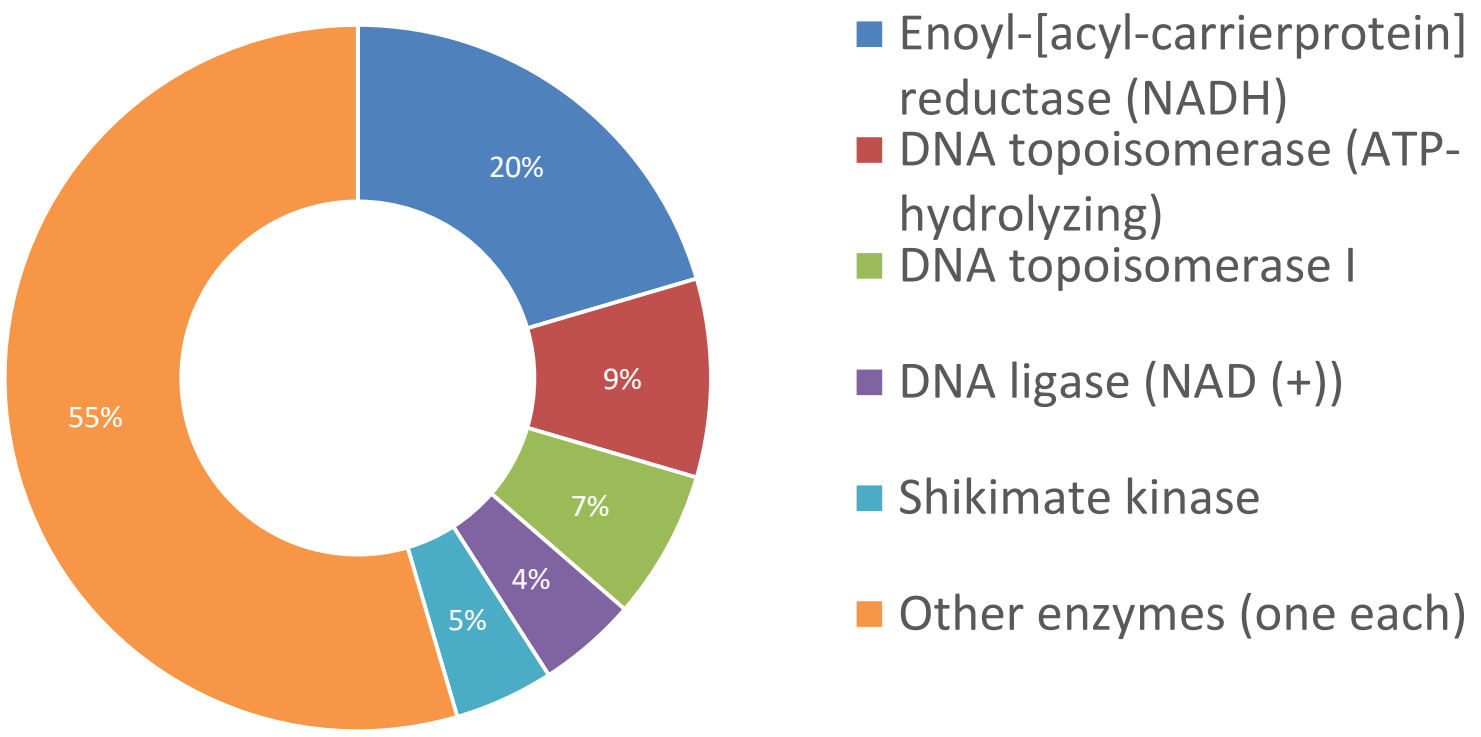
[5] Saxena, S., et al. Identification of novel inhibitors against Mycobacterium tuberculosis L-alanine dehydrogenase (MTB-AlaDH) through structure-based virtual screening. J. Mol. Graph. Model. 2014, 47, 37–43.
[6] Cinu, T.A., et al. Design, synthesis and evaluation of antitubercular activity of Triclosan 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



Results and discussion

1. *Mycobacterium tuberculosis* Enzyme Targets



Aditional 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

Results and discussion

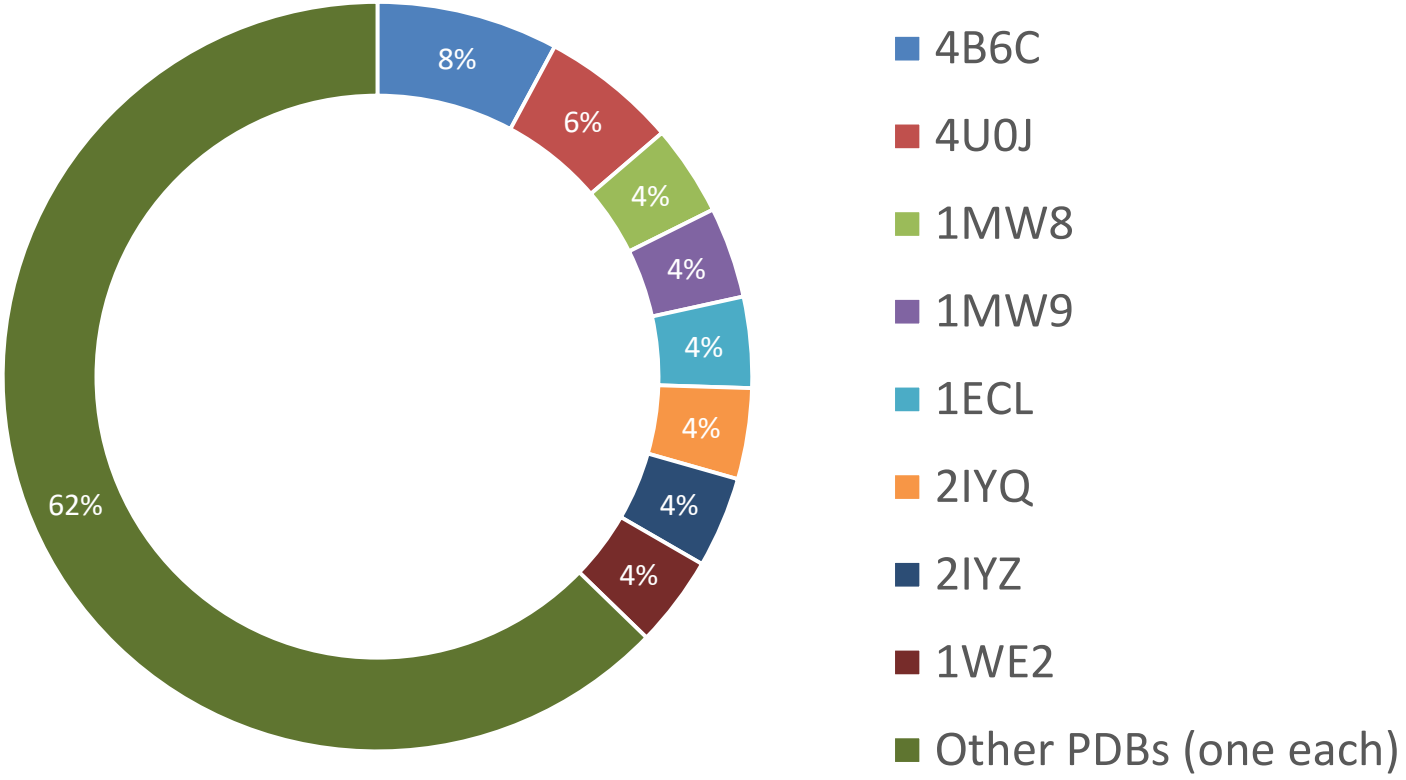
1. Mycobacterium tuberculosis Enzyme Targets

- 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



Aditional 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

Results and discussion

2. PDB

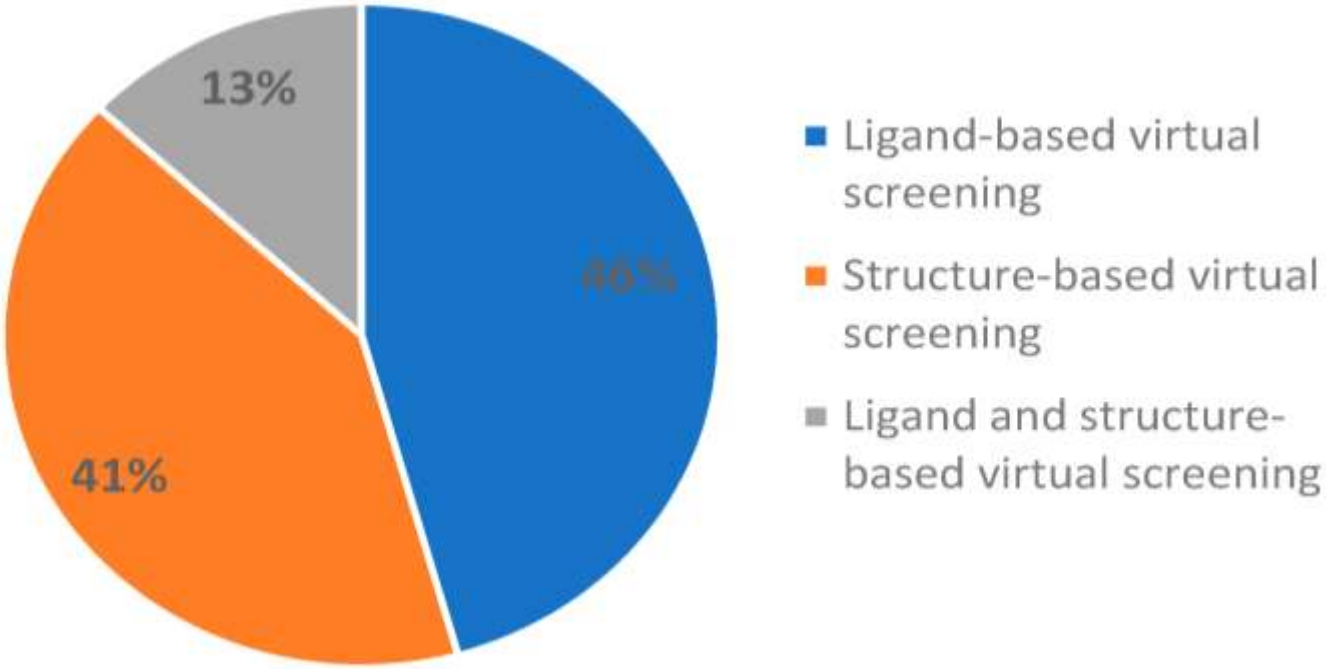
- We found **40** different PDBs analyzed within the 46 retrieved manuscripts.

- 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

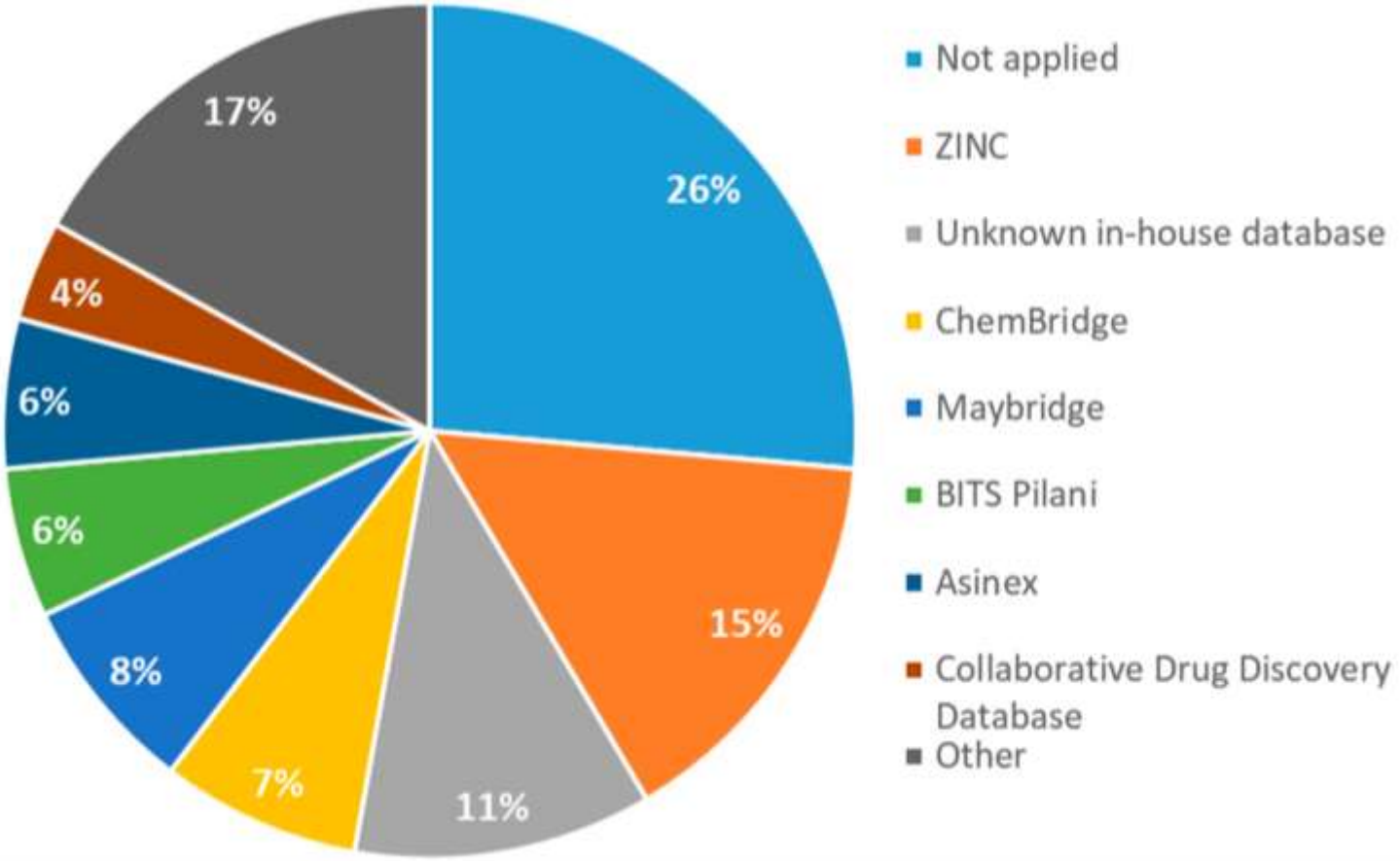
3. Virtual Screening Methods Applied

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



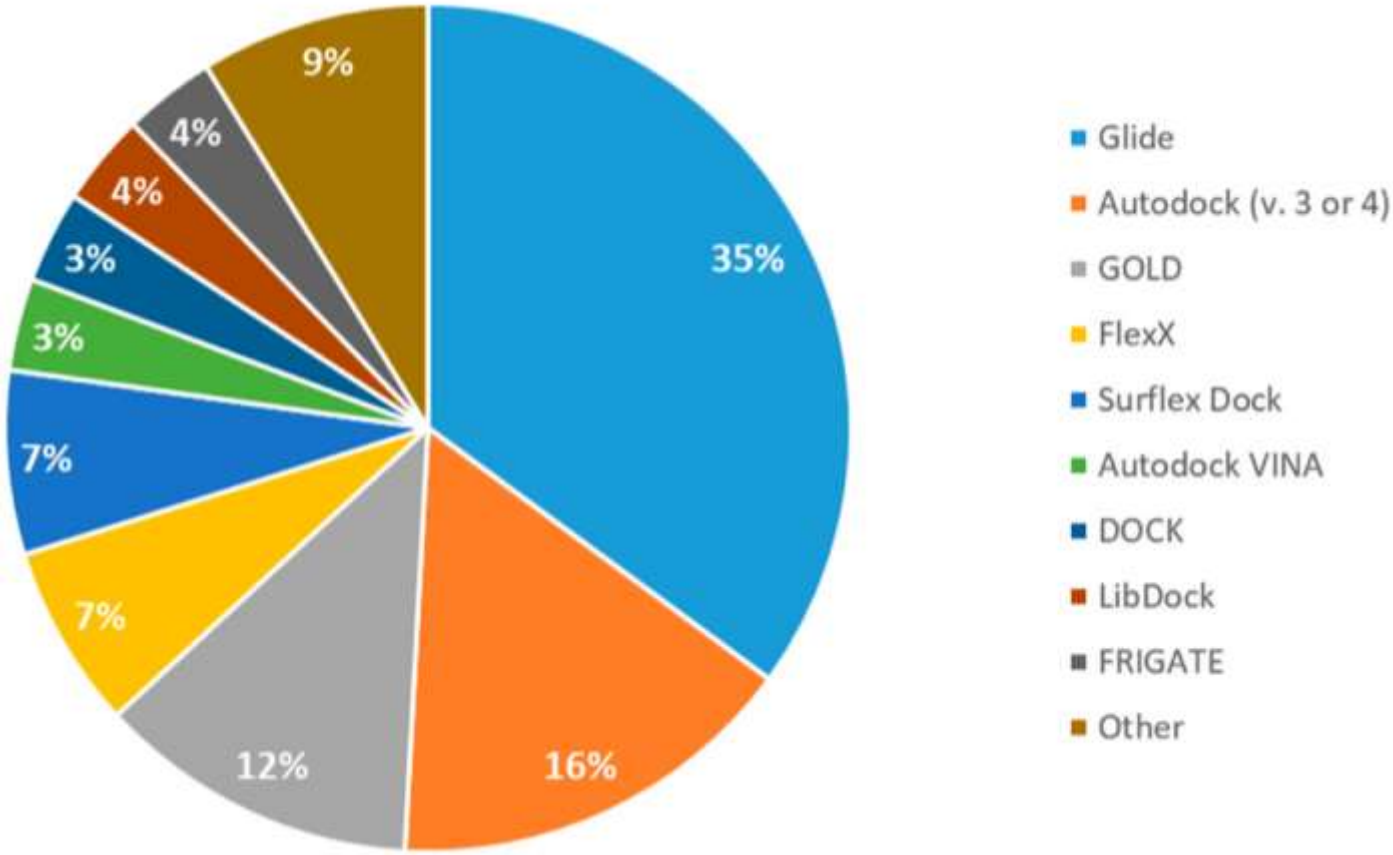
Aditional information and references are listed in Timo, G.O, et al. Predictive Power of In Silico Approach to Evaluate Chemicals against *M. tuberculosis*: A Systematic Review. *Pharmaceuticals* **2019**, 12, 135. DOI: https://doi.org/10.3390/ph12030135

Results and discussion
4. Databases Screened



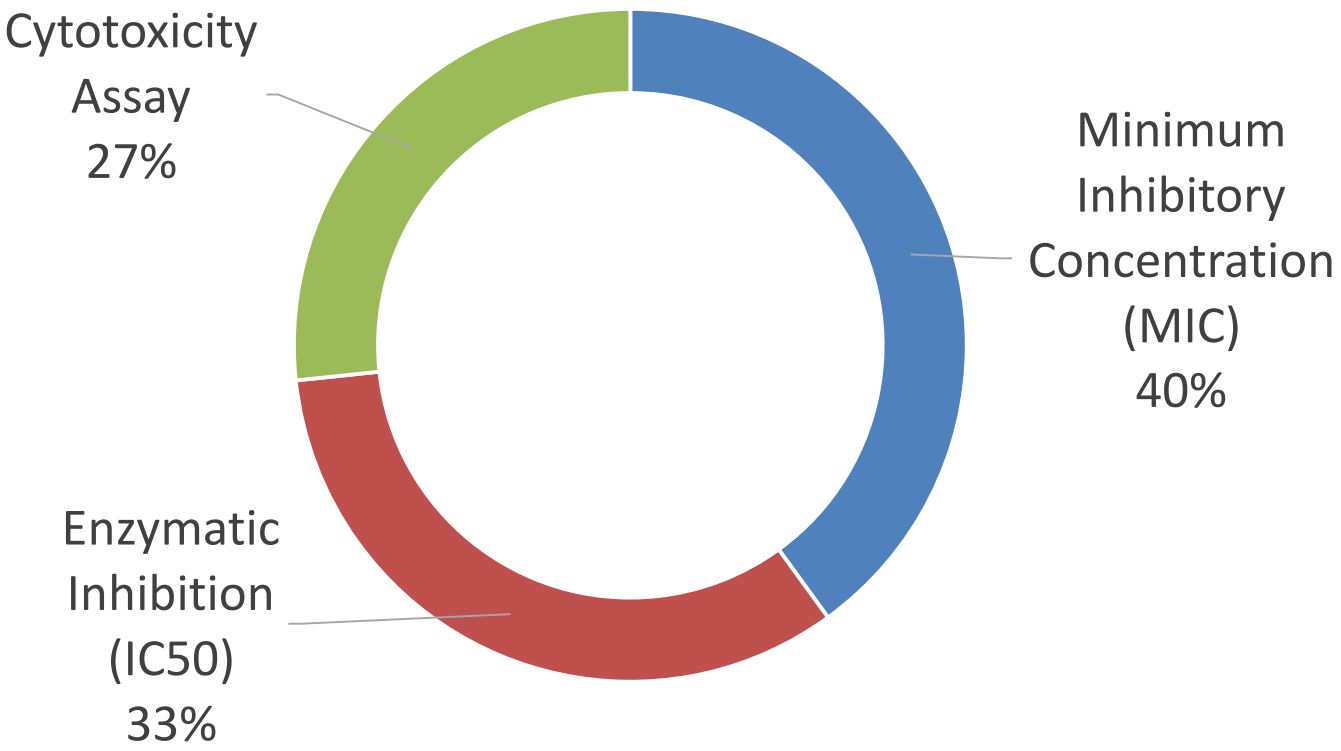
Aditional information and references are listed in Timo, G.O, et al. Predictive Power of In Silico Approach to Evaluate Chemicals against *M. tuberculosis*: A Systematic Review. *Pharmaceuticals* **2019**, 12, 135. DOI: https://doi.org/10.3390/ph12030135

Results and discussion
5. Docking Software Employed



Aditional 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

Results and discussion
6. In Vitro or In Vivo Testing



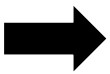
Aditional information and references are listed in Timo, G.O, et al. Predictive Power of In Silico Approach to Evaluate Chemicals against *M. tuberculosis*: A Systematic Review. *Pharmaceuticals* **2019**, 12, 135. DOI: https://doi.org/10.3390/ph12030135

Results and discussion

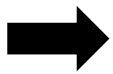
6. *In Vitro* or *In Vivo* Testing

- 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)



MIC = 0.78 µM

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.

$$\frac{MIC\ from\ new\ developed\ molecule}{MIC\ from\ standard\ approved\ drug}$$

- Molecules were considered excelent if they had MIC ratio below or close to **1** → 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.

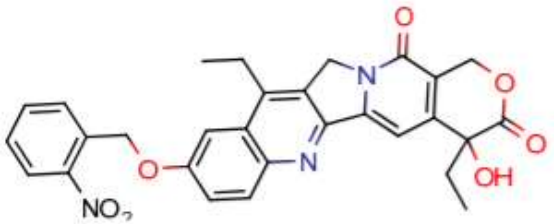
Extra information about compound nomenclatures and molecular structures, MICs, ratio values, IC_{50}, and docking scores are shown in Table 2 from Timo, G.O, et al. Predictive Power of In Silico Approach to Evaluate Chemicals against *M. tuberculosis*: A Systematic Review. *Pharmaceuticals* **2019**,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

Results and discussion

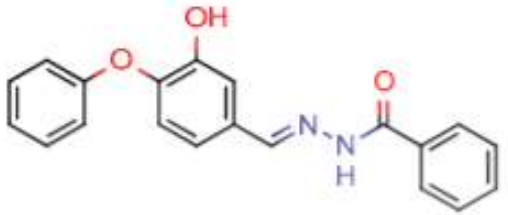
6. *In Vitro* or *In Vivo* Testing

- Molecular structures from compounds with best MIC ratio

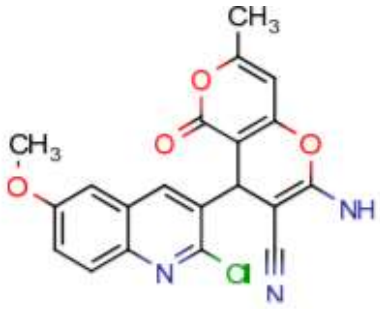
C9



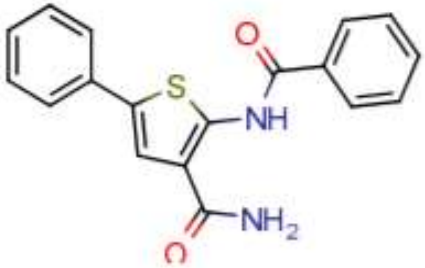
3b



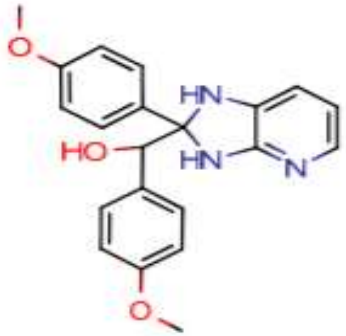
DE3



PA



23



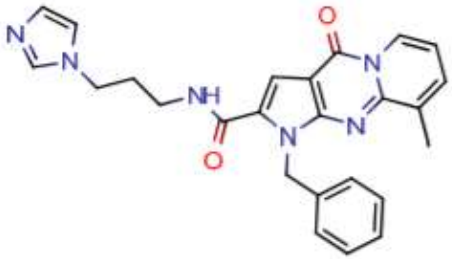
2j

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.

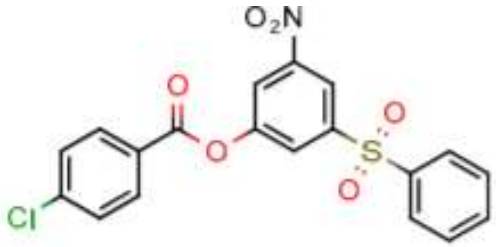
Results and discussion

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

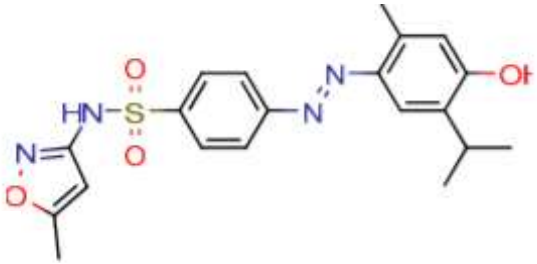
- Molecular structures from compounds with best MIC ratio



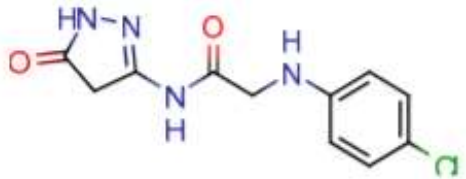
1



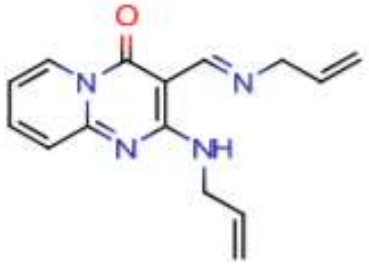
15



Conjugate-5



8b



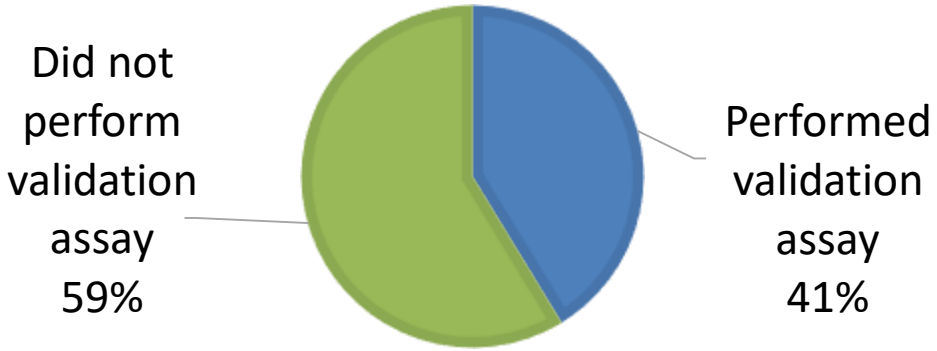
TB8

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.

Results and discussion

7. Validation procedures

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

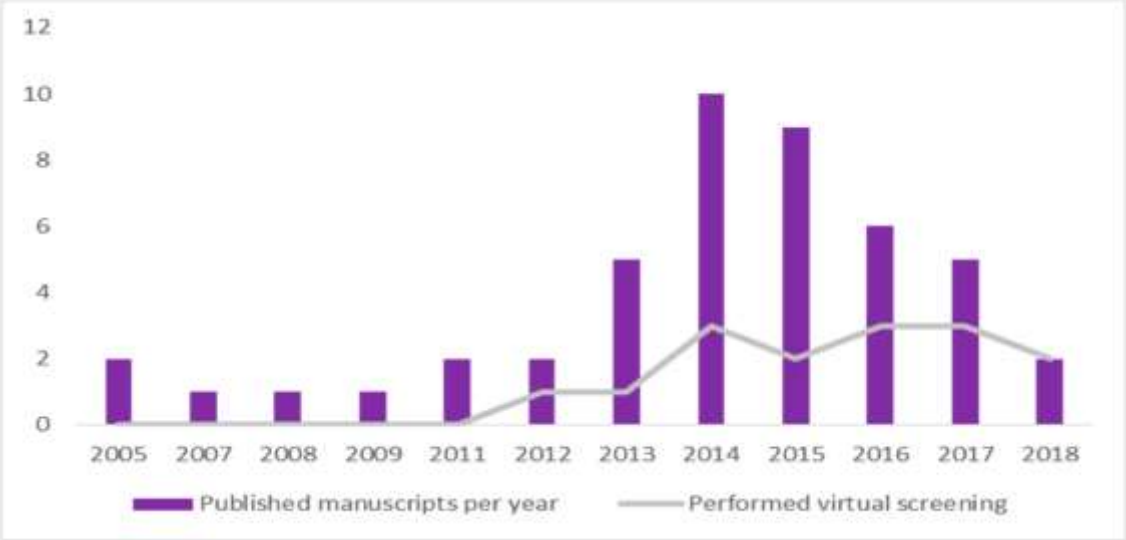


- Validations experiments considered:

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

M.H.-d.-M. and G.O.T. designed the study; G.O.T., R.S.S.V.d.R., A.F.d.M., and T.V.L.C. performed the searches; G.O.T. analyzed the data and wrote the paper; M.H.-d.-M. and P.d.O.M. supervised the process.

Also, we would like to thank Fundação de Apoio à Pesquisa do Distrito Federal (FAP-DF) for sponsoring our team and University of Brasília for providing structure and means for this research to happen.