molecular informatics

DOI: 10.1002/minf.200((full DOI will be filled in by the editorial staff))

SCREENING OF *Mycobacterium smegmatis* RNA POLYMERASE INHIBITORS THROUGH AN *in Silico* DRUG REPURPOSING APPROACH

Adriana Françozo Melo[a], Elizabete Cristina Iseke Bispo[a] Pérola de Oliveira Magalhães[b] and Mauricio Homem-de-Mello*[a]

Abstract: Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb) and is a worldwide problem. Treatment consists of a combination of antibiotics, one of them is rifampicin (RFP). This drug acts by inhibiting the bacterial RNA polymerase, an enzyme essential to mycobacterial survival, but subject to mutations that lead to resistance to RFP. Using an *in silico* drug repurposing approach, we have detected molecules already commercially available or that are in an advanced clinical development stage that are capable to interact with the bacterial RNA polymerase of *Mycobacterium smegmatis*, a PDB that have already shown good correlation with Mtb analogue protein. In this study, 20 molecules had satisfactory results for docking and 6 of them are candidates for further *in vitro* and/or *in vivo* testing. In addition, our study aims to reduce the costs and time of research for the development of new active drugs against *M. tuberculosis.*

Keywords: *Mycobacterium tuberculosis*; Tuberculosis; Drug Repurposing; Rifampicin; RNA Polymerase

1 Introduction

Tuberculosis (TB) is an infectious disease caused by the actinobacteria *Mycobacterium tuberculosis* (Mtb). In 2017, about 10 million individuals were infected, and 1.6 million died, thus being the world's most deadly infectious disease.[1]

Treatment of tuberculosis consists in a combination of drugs that usually are Rifampicin (RFP), Isoniazid (INZ), Pyrazinamide (PYR) and Ethambutol (ETH), but bacteria is creating mechanisms of resistance to those drugs. The regimen of new TB patients consists in 2 months of that combination and 4 months of RFP and INZ (2HRZE/4HR).[2].

RFP is an inhibitor of Mycobacteria DNA-dependent RNA polymerase.[3] The side effects include hepatotoxicity, dermatological reactions, neurological and gastrointestinal disorders.[4] Unfortunately, RFP resistance is arising day by day either by the difficulty of adhesion due to the adverse effects and long-time of treatment, or by mutation of the molecular target. The development of RFP resistance involves almost exclusively mutations of the central region of the gene encoding the β subunit of RNA polymerase (rpoB).[5] More than 96% of rifampicin-resistant strains have a mutation within this central region, composed of 81 base pairs. In this way, it is possible to identify resistant strains in a relatively simple way.[6]

Due to that situation, it is important to find drugs that are capable to inhibits the resistant bacteria. A trending approach is the *in silico* drug repurposing, using commercially available drugs. Virtual screening of already marketable molecules may improve clinical testing time, since toxicity and adverse effects profile are usually better known.[7] Thus, the aim of the study is to find molecules that may target the bacterial RNA polymerase enzyme using RFP as model of ligand.

2 Material and Methods

2.1 Ligand Library

Database obtained from Clarivate Analytics©, Integrity®, was used to virtual screening. Molecules were selected using "Therapeutic group" and "Development Phase" as filters. Therapeutics groups used to constitute the library were antiepileptics, antidepressants, anti-inflammatory and anxiolytic drugs. Integrity catalogues include all pharmacological properties already researched, so it is more inclusive than a regulatory information. Since our aim is drug repurposing, we filtered molecules that were Clinical Phase III onwards, as this choice is possible on Integrity. The resulting lists were then transferred to a Microsoft® Excel™ and used to construct a library by a search in ZINC and PubChem databases.

[a] *InSiliTox, Department of Pharmacy, University of Brasilia*
Campus Universitario Darcy Ribeiro, Health Sciences School
**e-mail:* mauriciohmello@unb.br, *phone:* +55 61 3107-1806
[b] *Natural Products Laboratory, Department of Pharmacy, University of Brasilia*
Campus Universitario Darcy Ribeiro, Health Sciences School

2.2 Virtual Screening

The software used for Virtual Screening was GOLD 5.7.0. Protein PDB used for the analysis was the *M. smegmatis* RNA polymerase 6CCV (R= 3.05 Å), that has already been used as a good equivalent to *M. tuberculosis* protein.[8]

RFP was excluded from the molecule, as well as waters, and all hydrogens were added. RFP binding domain was used as the coordinate to the software perform the interactions, in a radius of 10 Å. The chosen template was gold_kinase_VS, and the process was carried out using rigid protein and ligands. Following the recommendation of the guideline of GOLD,[9] the Scoring Function (SF) used was default CHEMPLP, and the configuration was to generate 10 docking poses for each drug. Results were then ranked by Fitness Score (FS).

2.3 Validation

Re-docking of the original RFP ligand was used for validation of the analysis. In this step, all ligand bonds flexibility and rotation were allowed. Into the binding site, the following amino acids were left free for rotation and flexibility as well: E481, I488, L454, L446 and Q429, identified as the main responsible for Van der Waals interactions with the ligand.[5] Amino acids identified as responsible for hydrogen bonds were left rigid, since its flexibility and/or rotation caused fatal errors during docking process. All ligands but a SO_4 close to the biding site were removed, as well as all water molecules. The SF used was CHEMPLP.

2.4 Docking

The selected ligands were then docked to RNA polymerase using full flexibility and rotational features. The protein, however had to be kept rigid because of several fatal errors during the runs. All other configurations were repeated in this step.

3 Results and Discussion

Tuberculosis is a worldwide problem,[10] and one of its standard treatments uses rifampicin as a antimicrobial agent.[11] The molecular target of rifampicin is the enzyme RNA polymerase, and its RFP binding site shows polymorphism that provides drug resistance.[5] Innovation in this field has not yet found really effective substances, and the search using *in silico* tools is a functional way in drug discovery.[12] In the present work, we used an interesting tool to select molecules already in advanced stage of clinical/pharmacological development, or already in the market, aiming to drug repurposing. This would bring cost and time advantages into the development of a new anti-tuberculosis chemical.[13]

3.1 Virtual Screening Results

Screening in Integrity provided 244 different drugs, within the selected categories. mol2 files were obtained in ZINC (231) or PubChem (13). Screening process finished with 479 best pose solutions that were ranked by highest FS.

Of all ligands, we selected the 20 best ranked according to FS results (Table 1) to go through docking analysis. This sampling represents 8.16% of all drugs tested during the virtual screening process.

Table 1. Virtual Screening best rank drugs

Molecule	Integrity Category	Database code	PLP.Fitness
Balixafortide	AI	91864511[a]	104.38
Zafirlukast	AI	896717[b]	81.34
Hesperidin	AD	8680006[b]	77.10
Astaxanthin	AI	17653967[b]	71.75
Ansofaxine	AD	15983287[a]	71.50
Risperidone	AD, AX	538312[b]	69.16
Zuranolone	AD	86294073[a]	68.96
Cyamemazine	AX	62865[a]	68.15
Apadenoson	AI	3966397[b]	68.12
Paliperidone	AD	1481956[b]	68.11
Padsevonil	AE	52911611[a]	67.88
Nefazodone	AD	538065[b]	66.74
Iloperidone	AD	1548097[b]	65.90
Pagoclone	AX	3775812[b]	65.15
Mifepristone	AX	14984307[b]	64.95
Vilazodone	AD	1542113[b]	64.38
Minocycline	AD, AI	16186307[b]	63.71
Lurasidone	AD	65743151[b]	63.70
Flibanserin	AD	52716421[b]	63.51
Casopitant	AD, AX	56898754[b]	62.92

[a] PubChem numbers.
[b] ZINC numbers
AE: antiepileptics, AD: antidepressants, AI: anti-inflammatory
AX: anxiolytic

All drugs from Table 1 were searched in Pubmed as "Drug name AND tuberculosis". Only zafirlukast[14] and hesperidin[15] showed one research aiming to evaluate this pharmacological property. Minocycline, as expected, is an antibiotic and had 79 hits, but only three[16-18] in last five years that investigated the drug efficacy against *M. tuberculosis*.

3.2 Validation

RMSD results of validation are presented in Table 2, representing the mean deviation of the tested structure pose in comparison to the original ligand pose. Figure 1 shows best ranked and original RFP poses. Results show that the method used in virtual screening for the selection of molecules was effective. GOLD Genetic algorithm considers that when two molecules share the same niche their RMSD is less than 1.0 Å.[9]

Table 2. Validation Results

Molecule	PLP.Fitness	RMSD	PLP.Fitness	RMSD
RFP	85.06	1.026	82.57	0.811
	84.63	1.137	82.41	0.924
	82.89	1.119	81.11	1.083
	82.74	1.007	80.48	0.998
	82.73	0.987	79.56	1.336

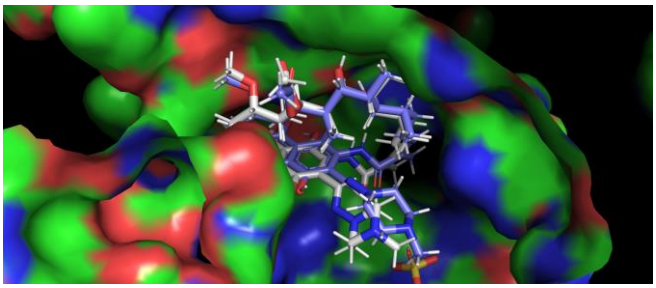


Figure 1. Validation results – Best ranked (gray) and original (blue) RFP poses. Figure obtained in PyMOL

3.3 Docking Results

All 20 best ranked molecules were docked flexible, obtaining more precise results, depending on the molecule diversity possibilities. We chose to best evaluate those molecules that presented PLP.fitness greater than 70, resulting in 6 molecules. Some of them had a FS greater than that of rifampicin itself (Table 3). These results show that the molecules tested are promising candidates for future in vitro tests, since PLP.Fitness is an indicative of interaction and fit between the ligand and the protein.[9] Among these drugs, four are already available in the market and two are in phase III of pharmacological development.

Table 3. Docking Results

Molecule	PLP.Fitness	Phase Development
Balixafortide	110,57	III
Astaxanthin	82,39	Launched
Zafirlukast	79,42	Launched
Hesperidin	77,64	Launched
Zuranolone	72,96	III
Nefazodone	70,15	Launched

Balixafortide is in Phase III of clinical development (Clinical Trial NCT03786094), and it is intended to be used in breast cancer, HER2 negative cases. Toxicological profile is still under evaluation but seems to be safer than the actual treatment.[19]

Astaxanthin (Figure 4A) is a carotenone compound, used as diet supplement, and registered in many countries. It is used mainly because of the antioxidant and anti-inflammatory effects. It is found in many aquatic organisms in red pigment.[20] The toxicological profile associated to astaxanthin is mainly due to its anticoagulant properties, and so there are possibilities of drug interactions with several therapeutic classes that present the same activity.[21]

Zafirlukast (Figure 4D) is a known leukotriene inhibitor, used for asthma and other pulmonary diseases. It has a high tolerability and a low toxicity profile as well. Several clinical studies were carried out using this drug.[22]

Hesperidin (Figure 4B) is a flavan-on glycoside found in citrus fruits, and it is known for its antioxidant and cardioprotective effects, even if it is still controversial.[23]

Zuranolone (SAGE-217) is a new antidepressant proposed to major and postpartum depression. It is a potent $GABA_A$ agonist[24] and still needs further investigation about its safety and efficacy.[25]

Nefazodone (Figure 4C) is an antidepressant used in several depression disorders. It is a Serotonin antagonist and reuptake inhibitor (SARI), and it has been well tolerated in clinical comparative studies.[26]

Balixafortide and Zuranolone characteristics signalize negatively towards a drug repurposing intention, even presenting a high PLP.Fitness. Astaxanthin, Zafirlukast, Hesperidin and Nefazodone shows a low toxicological profile and are somehow easy to obtain, thus been indicated as a potential candidate to *in vitro* testing against *M. tuberculosis*.

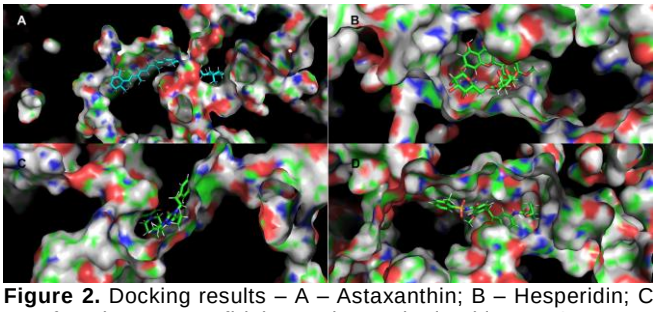


Figure 2. Docking results – A – Astaxanthin; B – Hesperidin; C – Nefazodone; D - Zafirlukast. Figure obtained in PyMOL

Innovation in tuberculosis therapy is hard to achieve. In recent years, only two new drugs (bedaquiline and delamanid) were approved to this use, but the old conventional treatment is still the gold standard in this therapy.[27] Use of computational tools and drug repurposing, using broader therapeutic classes would improve this search. This was the main limitation of this study, since we chose to be limited to fewer classes.

3 Conclusions

Integrity® database is an interesting tool in searching drugs according to their therapeutic or clinical status. Using a structure based virtual screening and a validated docking analysis, we found six potential ligands to RNA polymerase of a mycobacterium. Four of those are relatively safe and commercially available. Future *in vitro* studies are needed to confirm this potential activity, and this manuscript's intention is to provide new information in a still cloudy research field.

Acknowledgements

This work was supported by a grant from Fundação de Apoio à Pesquisa do Distrito Federal (FAP-DF) (process 0193.001797/2017).

References

[1] Engaging Private Health Care Providers In Tb Care And Prevention: A Landscape Analysis, Switzerland: *World Health Organization*; **2018**; p. 06 (https://www.who.int/tb/publications/2018/PPMLandscapeAnalysis.pdf, accessed 01 July 2019)

[2] Guidelines for treatment of tuberculosis, fourth edition. Geneva: *World Health Organization*; **2010**; p. 02 (http://www.who.int/tb/publications/2010/9789241547833/en/, accessed 01 July 2019)

[3] W. Lin, S. Mandal, D. Degen, *Mol. Cell*. **2017**, *66*, 1-11.

[4] O. E. Bouazzi, S. Hammi, J. E. Bourkadi, A. Tebaa, D. S. Tanani, R. Soulaymani-Bencheikh, N. Badrane, R. Bengueddour, *Pan African Med. Journal*. **2016**, *25*, 167.
[5] E. A. Campbell, N. Korzheva, A. Mustaev, K. Murakami, S. Nair, A. Goldfarb, S. A. Darst, *Cell Press*. **2001**, *104*, 901-912.
[6] A. Somoskovi, L. M. Parsons, M. Salfinger, *Respir. Res.* **2001**, *2*, 164-168.
[7] V. Vyas, A. Jain, A. Jain, A. Gupta, *Sci. Pharm.* **2008**, *76*, 333–360.
[8] J. Peek, M. Lilic, D. Montiel, A. Milshteyn, I. Woodworth, J. B. Biggins, M. A. Ternei, P. Y. Calle, M. Danziger, T. Warrier, K. Saito, N. Braffman, A. Fay, M. S. Glickman, S. A. Darst, E. A. Campbell, S. F. Brady, *Nat. Commun.* **2018**, *9(1)*, 4147.
[9] GOLD User Guide, 2019 CSD Release Update 2. *The Cambridge Crystallographic Data Centre (CCDC)*; **2019** (https://www.ccdc.cam.ac.uk/support-and-resources/ccdcresources/GOLD_User_Guide.pdf accessed 01 July 2019).
[10] R. Anand, *Interdiscip. Sci.: Comput. Life Sci*. **2016**, *10(2)*, 419-429.
[11] A. Chakraborty, A. K. Panda, R. Ghosh, I. Roy, A. Biswas, *Int. J. Biol. Macromol*. **2019**, *127*, 187-196.
[12] V. S. Pinto, J. S. C. Araújo, R. C. Silva, G. V. da Costa, J. N. Cruz, M. F. A. Neto, J. M. Campos, C. B. R. Santos, F. H. A. Leite, M. C. S. Junior, *Pharmaceuticals*, **2019**, *12*, 36.
[13] T. Cheng, Q. Li, Z. Zhou, Y. Wang, S. H. Bryant, *AAPS J.* **2012**, *14*, 133-141.
[14] L. Pinault, J. S. Han, C. M. Kang, J. Franco, D. R. Ronning, *Antimicrob. Agents Chemother*. **2013**, *57(5)*, 2134-40.
[15] Y. Zhao, Y. Jiao, L. Wang, *J. Spinal Cord. Med*. **2018**, *20*, 1-8.
[16] D. Deshpande, J. G. Pasipanodya, S. Srivastava, K. R. Martin, S. Athale, J. van Zyl, J.Antiabong, T. Koeuth, P. S. Lee, K. Dheda, T. Gumbo, *J. Infect. Dis.* **2019**, *219(6)*, 975-985.
[17] F. Bouzid, H. Astier, D. A. Osman, E. Javelle, M. O. Hassan, F. Simon, E. Garnotel, M. Drancourt, *Int. J. Antimicrob. Agents.* **2018**, *51(2)*, 235-238.
[18] L. Mangum, D. Kilpatrick, B. Stryjewska, R. Sampath, *Open Forum Infect. Dis.* **2018**, *5(7)*, 133.
[19] Polyphor Ltd., Pivotal Study in HER2 Negative, Locally Recurrent or Metastatic Breast Cancer, *NIH, U.S. National Library of Medicine*, December 24, **2018** (https://clinicaltrials.gov/ct2/show/NCT03786094 accessed 09 July 2019).
[20] S. J. Lee, S. K. Bai, K. S. Lee, S. Namkoong, H. J. Na, K. S. Ha, J. A. Han, S. V. Yim, K. Chang, Y.G. Kwon, S. K. Lee, Y. M. Kim, *Mol. Cells.* **2003**, *16(1)*, 97-105.
[21] K. C. Chan, P. J. Pen, M. C. Yin, *J. Food Sci*. **2012**, *77*, H76–H80.
[22] L. Aagaard, E. H. Hansen, *Int. J. Clin. Pharm.* **2014**, *36(2)*, 243-52.
[23] L. Pla-Pagà, J. Companys, L. Calderón-Pérez, E. Llauradó, R. Solà, R. M. Valls, A. Pedret, *Nutr. Rev.* **2019**. [Epub ahead of print]
[24] G. M. Botella, F. G. Salituro, B. L. Harrison, R. T. Beresis, Z. Bai, M. J. Blanco, G. M. Belfort, J. Dai, C. M. Loya, M. A. Ackley, A. L. Althaus, S. J. Grossman, E. Hoffmann, J. J. Doherty, A. J. Robichaud, *J. Med. Chem.* **2017**, *60(18)*, 7810-7819.
[25] Sage Therapeutics, A Study to Evaluate the Safety, Tolerability, and Efficacy of SAGE-217 Compared to Placebo in Adult Subjects With Comorbid Major Depressive Disorder and Insomnia, *NIH, U.S. National Library of Medicine*, December 11, **2018** (https://clinicaltrials.gov/ct2/show/NCT03771664?term=sage-217&rank=8 accessed 09 July 2019).
[26] A. Cipriani, X. Zhou, C. Del Giovane, S. E. Hetrick, B. Qin, C. Whittington, D. Coghill, Y. Zhang, P. Hazell, S. Leucht, P. Cuijpers, J. Pu, D. Cohen, A. V. Ravindran, Y. Liu, K. D. Michael, L. Yang, L. Liu, P. Xie, *Lancet*. **2016**, *388(10047)*, 881-90.
[27] L. D'Ambrosio, R. Centis, S. Tiberi, M. Tadolini, M. Dalcolmo, A. Rendon, S. Esposito, G. B. Migliori, *J. Thorac. Dis.* **2017**, *9(7)*, 2093-2101.

Received: ((will be filled in by the editorial staff))
Accepted: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))