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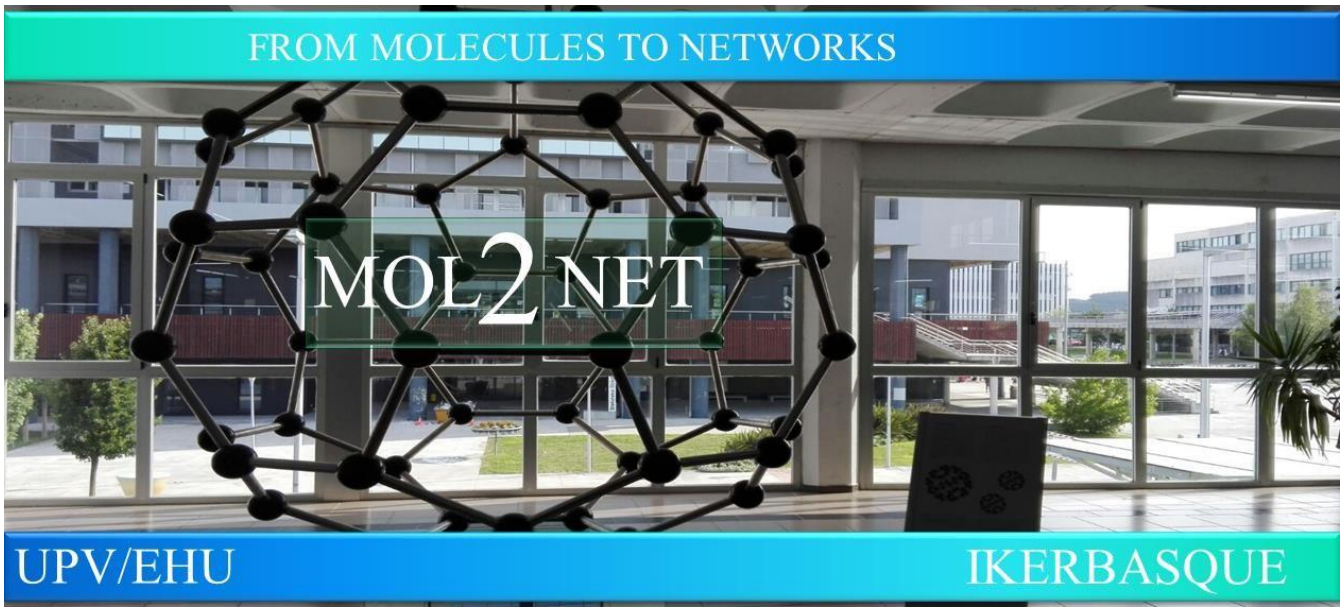

In silico study of potentials inhibitors of the enzyme shikimate kinase of *Mycobacterium tuberculosis* using molecular docking

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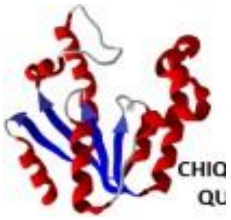
Graphical Abstract



Shikimate kinase

Abstract

Tuberculosis (TB) is an alarming infection worldwide, being one of the main causes of death by a single pathological agent, *Mycobacterium tuberculosis* (Mtb). Despite enormous research efforts on drugs for the treatment of tuberculosis, including vaccines and diagnostic investigations, this disease is still a major public health problem. Thus, for the treatment of tuberculosis, a new molecular design is needed in order to improve the current treatment and bypass Mtb resistancemechanisms.

Introduction

Since its discovery in 1882, the so-called Koch's bacillus (Mycobacterium tuberculosis, Mtb) has never ceased to affect humanity [1]. In 2020, tuberculosis (TB) became the second leading cause of death in the world from a single pathological agent, Mycobacterium tuberculosis (Mtb), second only to COVID-19. With the COVID-19 pandemic, there was an increase in the number of deaths from tuberculosis due a lack of access to diagnosis and treatment, a fact that occurred for the first time in ten years [2]. Furthermore, the emergence of drug-resistant strains of TB makes urgent the search for less toxic drugs and efforts to improve current treatment and bypass Mtb resistance mechanisms [1].

The shikimate pathway, which is present in bacteria, fungi and plants but absent in humans, has been important for the development of new anti-TB therapeutic agents. [3]. The enzyme shikimate kinase (SK) is a member of the Nucleoside Monophosphate Kinase (NMP) family, an important group of enzymes that catalyze the reversible transfer of a phosphate from a nucleoside triphosphate to a specific nucleoside diphosphate. This enzyme catalyzes the fifth step of the shikimate pathway, which is shikimate phosphorylation (SKM), using ATP as a phosphate donor to form shikimate-3-phosphate (S3P) and adenosine diphosphate (ADP). Based on the determination of the SKM binding site in a crystallographic structure of SK complexed with ADP:SKM and the structure ATP:shikimate 3-phosphotransferase, it was possible to have a better understanding of the intermolecular interactions between the ligands and the enzyme [4].

In order to assist in the development of new drugs, computational tools can be used, as they facilitate the detailed understanding of protein-ligand interactions. Therefore, in this work, we used docking simulations to identify potential MtSK inhibitors from the library of molecules synthesized by the Research Center for Molecular and Functional Biology (CPBMF), Brazil. Compounds that showed the best binding energy predicted by docking simulations were subjected to in silico prediction of toxicity and hepatotoxicity using pkCSM [5]. Thus, the results obtained serve as a basis for further efforts aimed at designing new anti-TB agents, as well as potential MtSK inhibitors.

Materials and Methods

The crystallographic structures of the MtSK protein were obtained from the Protein Database (PDB) (http://www.pdb.org, accessed on: June 24, 2021). Among the 21 available MtSK structures, 12 compounds showed interactions with the active site of the SKM substrate (PDB accession codes: 4BQS, 2IYQ, 2IYS, 2IYR, 2G1K, 2IYX, 2IYY, 2IYZ, 2DFN, 1U8A, 1ZYU and 1WE2). For docking, the following structures were selected: 2IYQ [6] (structure with closed LID, MtSK:SKM:ADP complex, 1.80 Å resolution) for its high resolution (<2 Å) and for its closed conformation, and 2IYS [6] (structure with open LID (A), MtSK:SKM complex, resolution of 1.40 Å) because it is the best resolution among all crystallographic structures deposited and because it contains shikimate in the active site of the structure.

The compounds tested in this work were synthesized by the CPBMF of the Pontifical Catholic University of Rio Grande do Sul, Brazil. The pkCSM machine learning platform [5] was used for a preliminary analysis of the toxicity of these compounds. A set of 1212 molecules passed through the AMES toxicity filter to select molecules without toxic or hepatotoxic character for subsequent computational simulations. From the screening, 298 molecules were selected for docking. The 3D structures of the compounds from the CPBMF database were designed using the Avogadro v1.2.0 program and the protonation states were defined according to pH 7.4.

Initially, the re-docking protocol was validated with two MtSK crystallographic structures. SKM was removed from both structures generating two different conformations: one containing ADP in the active site and the second in the apo form (PDB ID 2IYQ and 2IYS, respectively) [6]. The objective of performing this step was to determine if the docking algorithm can recover the crystallographic position of the ligand in the active site of the target protein.

Molecules from the CPBMF chemical library were donated into 2IYQ and 2IYS using the AutoDock Vina 1.1.2 software compiled for PyRx to identify the ligands with the best binding energies in relation to the target protein [7]. To complete the robustness of the method, the Lamarckian genetic algorithm was attributed, which is a computational approach that provides a set of potential conformations of ligands according to the principles proposed by Darwin during the molecular adjustment process [8]. PyRx was used for compound energy minimization and to convert all molecular tracks to AutoDock Ligand format (PDBQT). The established fitting protocol was carried out as follows: the SK was kept rigid, while the torsion angles of the binder could be varied. Water and other compounds used to obtain the crystal were removed from each frame prior to fitting.

The 2IYS and 2IYQ structures were used as macromolecules (receptors) [6]. The search space that encompassed the SKM active site was defined in a cubic grid, spaced at 0.375 Å, (in both crystallographic structures) with the following dimensions in Å: center (x, y, z) = (36, 36, 30), dimensions (x, y, z) = (15, 15, 15) (PDB: 2IYQ); center (x, y, z) = (15, 20, 33), dimensions (x, y, z) = (15, 15, 15) (PDB: 2IYS).

The fitting simulation was then run at an exhaustiveness of eight and set to produce a pose arrangement (from lowest to highest energy). Subsequently, these ligands were submitted to the PyMOL software (The PyMOL Molecular Graphics System, version 2.5 Schrödinger, LLC, https://www.pymol.org) and the Discovery Studio Visualizer program (CDOCKER Dock, Dassault Systemes BIOVIA, USA) for inspection. visual of the poses of potential ligands in the active site of the protein in both conformations.

Results and Discussion

The first criteria for selecting compounds from the internal chemical library were employed to filter AMES toxicity and hepatotoxicity. A set of 1212 chemical structures was submitted to the pkCSM platform [5] and 298 molecules passed through the AMES toxicity filter. Although these chemical compounds were not intentionally developed to bind MtSK, it was considered appropriate to assess whether or not bound to this enzyme could be identified among the chemical library.

AutoDock Vina 1.1.2 implemented in PyRx software [7] was used for re-docking and docking simulations. The Root Mean Square Deviation (RMSD) value between the ligand present in the crystalline structure and the fitting pose was 0.77 Å for 2IYS [6] and 0.66 Å for 2IYQ [6]. This result indicates that the docking simulation was able to reproduce a crystallographic pose and that the protocol is suitable to be used in virtual screening efforts.

The docking results were evaluated according to the best predicted binding affinity between the two crystallographic structures and these molecules. After simulations and visual analyses, 30

compounds with the best score were selected from the pool of 298 compounds. The top 30 candidate ligands were found to produce strong affinities ranging from -8.5 to -9.2 kcal/mol based on docking studies, and none of the 30 compounds analyzed exhibited AMES toxicity or hepatotoxicity.

From the visual analysis of the intermolecular interactions in the Discovery Studio Visualizer software, it was possible to observe the potential interaction of the ligands in the shikimate binding cavity. Knowledge of the amino acid residues involved in the protein: ligand interaction of the shikimate binding cavity highlights potential ligands with high binding affinity to this site and points out candidates for inhibitors. In addition, the residues that establish these molecular interactions are fundamental for the reaction catalyzed by the enzyme. The results showed that the ligand with the highest predicted binding affinity with the enzyme performs van der Waals-like interactions with some amino acid residues in the shikimate binding pocket. Furthermore, some amino acid residues have been shown to participate in intermolecular hydrogen bonds with shikimate [5].

By analyzing the potential ligands classified by binding energy, it is possible to observe essential residues that enable the binding of the ligand to the active site of the MtSK enzyme, mainly hydrogen bonds and van der Waals interactions.

Biochemical assays were used to provide experimental evidence for in-depth predictions in silico. The results obtained by the computational methodology were in good agreement with the experimental data, demonstrating that from 1212 structures it was possible to identify five compounds that inhibit both MtSK and the in vitro growth of Mtb, named 1a, 1b, 3a, 3d and 5c, and a compound that showed no enzymatic inhibition, but was active against the growth of mycobacteria named as compound 7b.

In summary, the obtained results show that the computational approach employed can identify ligands and a limited number of MtSK inhibitors from a very small library of chemical compounds, which represents a remarkable achievement. It is anticipated that this in silico approach will be employed shortly to screen much larger libraries of chemical compounds, ideally with price scaffolds covering a larger chemical space.

Conclusions

In this study, a proprietary chemical library containing different chemical classes was used to evaluate its binding and inhibition profile against the MtSK enzyme. Computational methodologies were used to evaluate the toxicity/hepatotoxicity of the molecules and to probe the intermolecular interactions of these compounds in the active site of the enzyme. In addition, biochemical assays were used to provide experimental evidence for in silico predictions. The results obtained should contribute to efforts to track inhibitors of the MtSK enzyme activity from, for example, structural modifications of the identified compounds in order to optimize their inhibitory activity against the MtSK enzyme and Mtb growth.

References

[1] C. L. Meena, P. Singh, R. P. Shaliwal, V. Kumar, A. Kumar, A. K. Tiwari, S. Asthana, R. Singh, D. Mahajan. Synthesis and evaluation of thiophene based small molecules as potent inhibitors of Mycobacterium tuberculosis. European Journal of Medicinal Chemistry, 208 (2020) 112772, https://doi.org/10.1016/j.ejmech.2020.112772.

[2] R.G. Ducati, L.A. Basso, D.S. Santos. Mycobacterial Shikimate Pathway Enzymes as Targets for Drug Design. Curr Drug Targets 8 (2007) 423–435, https://doi.org/10.2174/138945007780059004

[3] T. Parish, N.G. Stoker. The common aromatic amino acid biosynthesis pathway is essential in Mycobacterium tuberculosis. Microbiology (Reading) (2002) 3069-3077, https://doi.org//10.1099/00221287-148-10-3069

[4] J.D. Coracini, W.F. de Azevedo. Shikimate kinase, a protein target for drug design. Curr Med Chem. 21 (2014) 592–604, https://doi.org//10.2174/09298673113206660299

[5] D.E.V, Pires, T.L. Blundell, D. Ascher. BpkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. Journal of Medicinal Chemistry, 58(9), (2015), 4066–4072, https://doi.org//10.1021/acs.jmedchem.5b00104

[6] M.D. Hartmann, G.P. Bourenkov, A. Oberschall, N. Strizhov, H.D. Bartunik. Mechanism of phosphate transfer catalyzed by shikimate kinase from Mycobacterium tuberculosis. J Mol Biol 364 (2006) 411-423, https://doi.org//10.1016/j.jmb.2006.09.001

[7] S. Dallakyan, A. J. Olson. Small-Molecule Library Screening by Docking with PyRx. Chemical Biology, (2014). 243–250, https://doi.org//10.1007/978-1-4939-2269-7_19

[8] J. Fuhrmann, A. Rurainski, H.P. Lenhof, D. Neumann. A new Lamarckian genetic algorithm for flexible ligand-receptor docking. Journal of Computational Chemistry, NA–NA (2010), https://doi.org//10.1002/jcc.21478