Virtual screening of Leishmanial pyridoxal kinase enzyme inhibitors by repurposed anti-Trypanosomal libraries reveals two core scaffolds

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Abstract

Leishmania is an intracellular protozoal infection, and it is classified as a neglected disease by the World Health Organization (WHO). Annually more than 2 million newly diagnosed cases were treated with highly toxic drugs. Leishmanial pyridoxal kinase enzyme (LPDxK) is an essential and druggable target. DNDI1103666 is the most promising lead as a potential inhibitor for LPDxK.

Introduction

Leishmania is an intracellular protozoal infection, and it is classified as a neglected disease by the World Health Organization (WHO), and its ending is one of the sustainable development goals for the next decade.[1]. Annually more than 2 million newly diagnosed cases are affected by the three clinical forms of the disease: Cutaneous Leishmaniasis, mucocutaneous Leishmaniasis, and visceral Leishmaniasis[2].

The disease forms are differently treated by highly toxic drugs, mostly repurposed of already marketed drugs (pentavalent antimonials, amphotericin B, paromomycin, and miltefosine). There is a shortage of safe, short-course, and oral antileishmanial since the 1950s[3].

Macronutrients and micronutrients are essential for the survival of any living organism[4]. Sodium stibogluconate is a carbohydrate derivative that is reported to inhibit glycolysis and macromolecules synthesis in Leishmania[5].

Vitamin B6 (pyridoxal-5-phosphate) is an essential micronutrient playing in several metabolic reactions as a co-factor in transamination, racemization, decarboxylation, and elimination reactions of amino acids. Moreover, it also plays a role in the neurotransmitters' biosynthesis and folding of pyridoxal enzymes[6]. In contrast to most eukaryotic organisms, including Homo sapiens, which are synthesizing vitamin B6 by two

well-known de novo pathways, which are 1-deoxy-d-xylulose 5-phosphate (DOXP) dependent and DOXP independent, Leishmania is harnessing its micronutrient the vitamin B6 by salvage of its vitamers pyridoxal (PL), pyridoxamine (PM) and pyridoxine (PN) from the host environment[7][8].

Pyridoxal kinase enzyme (PDxK) catalyzes the formation of the biologically active form of vitamin B6 as a co-factor by the phosphorylation of B6 vitamers PL, PN, and PM into pyridoxal-5-phosphate (PLP). The phosphorylated PN and PM are enzymatically converted into PLP[9]. Moreover, Leishmanial PDxK is involved in the resistance to miltefosine, which is an oral chemotherapeutic agent used in the treatment of visceral Leishmaniasis[10].

Hence Leishmanial pyridoxal kinase enzyme (LPDxK) is an essential and druggable target for the Trypanosomatidae family[11][12][7], is targeted in this study and virtually screened by a library of 5587 compounds to discover potential inhibitors.

Molecular docking used for virtual screening, and the best scoring 1090 compounds are clustered to ensure structural diversity. The best 66 compounds analyzed for their pharmacokinetic and toxicological properties and the best five lead like candidates are selected for the molecular dynamics simulation study. Retro-synthesis of the lead compounds showed the possibility of cheap production because of the fewer organic synthesis steps.

Methods

Computational details

All computational tasks were performed on Intel (R) Core (TM) i5-6267U CPU @ 2.9 GHz processor with a memory of 8 GB ram running on a 64 bit Catalina and windows ten operating systems.

Molecular docking

Molecular docking is a valuable tool in drug design and discovery, and it is used to determine the best poses and fitting of ligands with their macromolecule target and to rank best complexes; moreover, it is a cost-effective alternative for High throughput screening (HTS) by performing virtual screening[13].

Two libraries of DNDI were obtained from ChEMBL - Neglected Tropical Disease archive and combined as a one library resulting in 5587 compounds. The recently resolved crystal structure of Leishmanial pyridoxal kinase enzyme (PDB code: 6k92) was protonated, automatically built, and connected by MOE 2015.1 software[14], and all water molecules

were removed. The active site was determined by the site finder option by MOE software. The ligand placement done by triangle matcher and London dG was used as a scoring function while the refinement method was performed as a rigid receptor with GBVI/WSA dg as a scoring function. The initial screening was performed by taking one pose and five refinements. The best five lead compounds were docked with Human pyridoxal kinase enzyme (PDB code: 3keu) and Trypanosomal pyridoxal kinase enzyme (PDB code: 3zs7). In addition to the High-Speed α shapes algorithm by MOE, The Genetic algorithm by Gold of CCDC[15] and Monte Carlo algorithm by XP Glide docking for extra precision (Glide, Schrödinger, LLC, New York, NY, 2017) with CHEMPLP and Glide score as scoring term respectively, were used to validate the results of the best five lead compounds.

Prime MM-GBSA simulation

The free energy of binding for the best five lead compounds complexes with LPDxK was calculated employing molecular mechanic-generalized Born surface area (MM-GBSA) integrated with Prime (Maestro v11.2, Schrodinger, LCC, New York, NY, 2017). The solvation model was VSGB, and the utilized force field was OPLS3.

Clustering

To ensure molecular diversity, Canvas was used (Canvas, Schrödinger, LLC, New York, NY, 2017). In which the Tanimoto coefficient is employed to calculate fingerprint-based molecular similarity, and dendritic hierarchal clustering was performed using the Kelley criterion.

Pharmacokinetic & Toxicological analysis

The resulting clusters are analyzed by Datawarrior by OSIRIS to eliminate the predicted mutagenic, tumorigenic, reproductive, and irritant compounds[16]. The best candidate in each cluster was selected according to its drug-likeness score.

SwissADME was used to filter the compounds from the previous step[17]. Those with the least Leadlikeness violations, in addition to those which were not violating Lipinski, Ghose, Veber, and Muegge, and pass the blood-brain barrier (BBB) without being a substrate for P-glycoprotein (Pgp) are selected.

SAR analysis

The core-based SAR analysis tool of Datawarrior software is used to analyze the

structure-activity relationship (SAR) of the lead compounds[16].

Molecular Dynamics

Molecular dynamics is used to simulate the induced fit of ligand-protein complexes in a physiological environment and check the stability of the complexes over the simulation period after energy minimization with RMS gradient 0.05. Equilibration has been done for 100 picoseconds (ps) at 300k; then, the simulation is started for 10000 ps at raised body temperature 312 k (39 C) using the MMFF94x force field and solvated in a cube of TIP3P water model at a margin of 6 Å. The used algorithm is NPA with NVT canonical ensemble. MOE 2015.1 software is used to run the simulation. Trajectories were analyzed by VMD 1.9.3, while Microsoft Excel was used to plot the RMSD, RMSF, and potential energy results.

Retro-synthesis

Spaya is used to predict the ease of organic synthesis by choosing the least possible steps to make the desired compound with the highest scoring routes[18].

Figure 1: Virtual screening workflow

Results and Discussion

Kinetoplastida Trypanosomatids are having an iconic group of neglected infectious diseases, which are Leishmanias, Chagas disease, and African sleeping sickness [19]. Hence two anti-trypanosomal libraries are investigated in a quest to discover an antileishmanial drug. The workflow was started with 5587 compounds, resulting in 1090 hits scoring above a threshold of -7 (kcal/mol), as shown in (Figure 1). To ensure hits diversity, the fingerprints of the top-scoring compounds in virtual screening were calculated by the Tanimoto coefficient and by using Kelley criterion to keep clusters highly populated with the smallest possible spread of its members[20][21], 73 clusters were resulted according to structural similarity. One representative compound per cluster was selected and subjected to in silico based fingerprint analysis to predict the pharmacokinetic and toxicological descriptors, as shown in (Figure 2). The clustering result and the dendrogram are in supplementary files.

Most of the synthesized drugs are failing because of toxicity in preclinical and clinical phases, and the main problem of current antileishmanial drugs is toxicity and long term courses of treatment[22][23]; therefore, any warning in the screening phase is helpful to ensure the safety of the final compounds. Since most neglected diseases are in developing countries, and most available drugs are intravenously administered, an orally administered drug with high bioavailability and a large volume of distribution is favorable[24]. Therefore by applying these pharmacokinetic descriptors in filtering, 20 candidates have resulted. Furthermore, by employing the Lead-likeness criterion with one or zero violation, the number of candidates dwindled to five, as shown in (Table 1). To ensure selectivity, the final compounds with two negative controls, triamterene and lidocaine, and the natural substrate vitamin B6 were docked with Human PDxK and Trypanosomal PDxK, and they were scoring lower than LPDxK[25] as shown in (Figure 3).

Figure 2: The relevant pharmacokinetic descriptors of compounds that resulted from clustering.

Table 1: Lead compounds and their descriptors.

Compound	S Score (kcal/mol)	MW (g/mol)	Consensus LogP	Estimated LogS	PSA (Å)	Lead-likeness violations
DNDI1103666	-7.8883	331.45	3.19	-3.53	50.53	1
DNDI66684	-7.8596	406.26	3.26	-4.64	62.72	1
DNDI96491	-7.8058	322.36	3.03	-4.43	72.53	1
DNDI160055	-7.6273	327.42	3.95	-4.7	66.74	1
DNDI64402	-7.5648	297.35	2.93	-3.74	53.08	0

Figure 3: Binding energies calculated by MOE 2015.1 for 1: DNDI1103666, 2: DNDI66684, 3: DNDI96491, 4: DNDI160055, 5: DNDI64402 NC1: negative control 1 is triamterene, NC2: negative control 2 is lidocaine.

To rationalize the differences in binding energies, MOE, Maestro, and PLIP web server are used to examine the interaction of protein-ligand complexes [26].

The compounds-enzyme interaction reveals more than four H-bond on average, while indirect H-bonding mediated by a water bridge cannot be excluded.

The reported primary active site amino acids involved in aiding natural substrates' interaction and catalysis are Ser12, Val19, Val41, Leu43, Ser47, Ile52, Arg56, Tyr85, Asn87, Asp124, Arg127, Asn151, Tyr152, Lys187, Ser188, His222, Arg225, Tyr226, the conserved GTGD 228-231 motif, Gln258, Ile261 and Ser277[7]. The molecular docking and MD simulation revealed new additional amino acids, which are Asp119, Asp125, Tyr129, and Thr227, are involved in ligand-active site interaction while all ligands were interacting with Gly228.

DNDI1103666 is the highest scoring in all applied methods (S score, ChemPLP, Glide XP score, and MM-GBSA) because of the ability of the both protonated nitrogen atoms in the side chain to form two salt bridges with Asp124 and Asp125 as shown in (Figure 4A), which is the most robust interaction and the expected increase the potency is 300 fold per salt bridge[27].

The DNDI66684, DNDI96491, and DNDI160055 are showing at least one agreement in the ranking by the applied scoring methods.

DNDI66684 ranking as the 2nd highest in S score and ChemPLP due to Piperazine's ability to form a salt bridge with either Asp124 or Asp231, as shown in (Figure 4B). The compound DNDI96491 ranked as the 3rd highest in S score, while Glide XP and ChemPLP scores agreed as the lowest ranking compound. This discrepancy maybe because of the deleterious effect of the freely rotating ethyl group, which decreases drug potency by 140 fold since the restriction of the conformational freedom is energetically unfavorable, albeit the presence of π - π stacking and π -cation interaction[27] While DNDI160055 is ranked 4th in S score and it is showing agreement in ranking between ChemPLP and MM-GBSA scores. The analysis of ligand-protein interaction reveals no salt bridge is formed between DNDI160055 and LPDxK. Despite the imidazole ring ability to form a salt bridge with Asp124 in compound DNDI64402, it scored the lowest S score and showed no agreement in the ranking by the other scoring methods. Again the ethyl rotation barrier is a plausible culprit in recorded ranking discrepancy.

In MD simulation, the protein backbone–individual inhibitor's Root Mean Square Deviation (RMSD) and the Root Mean Square Fluctuation (RMSF) in the individual amino acid side chain and ligand interaction were calculated over the time of the simulation, 10000 ps as shown in (Figure 5 and 6). The temperature, pressure, volume, and potential energy of the complexes remained relatively constant over simulation time, indicating the robustness and reliability of the MD simulation, as shown in (Figure 7). During the entire simulation, the RMSF was below 3.5 Å, except the DNDI160055 – protein complex, which shows relatively higher fluctuations, indicating a lower degree of conformational changes in the side chains, i.e., better stability. The mean RMSD value of all complexes was below 1.6 Å, denoting the stability of the macromolecular structure of the complexes over the simulation time. All complexes showed relative overlap except DNDI160055, which started to diverge after more than 7000 ps are passed of the simulation time.

Oxazole based compounds have been reported as a preclinical antileishmanial candidate that supports the phenyloxazole findings[28]. To my knowledge, this is the first report for phenoxy pyridine derivatives as a potential antileishmanials, as shown in (Table 2). The retro-synthesis of the lead compounds showed the possibility of organic synthesis with two steps on average. The lower synthesis steps ensure the lower costs for mass production for developing countries with neglected diseases[29][30].

Figure 4 A) schematic diagram of detailed protein-ligand (DNDI1103666) interactions after molecular docking. B) 3D representation of the binding pose of DNDI66684 showing salt bridges in yellow spheres connected by dashed lines, H-bonds in solid blue lines, and hydrophobic interactions in gray dashed lines.

Figure 5: RMSD plot (DNDI1103666-LPDxK, DNDI66684-LPDxK, DNDI96491-LPDxK, DNDI160055-LPDxK, and DNDI64402-LPDxK complexes).

Figure 6: RMSF plot of protein alpha carbons in DNDI1103666-LPDxK, DNDI66684-LPDxK, DNDI96491-LPDxK, DNDI160055-LPDxK, and DNDI64402-LPDxK complexes.

Figure 7: the potential energy plot of DNDI1103666-LPDxK, DNDI66684-LPDxK, DNDI96491-LPDxK, DNDI160055-LPDxK, and DNDI64402-LPDxK complexes during MD simulation at 312K.

Table 2: The SAR of lead compounds reveals the phenyloxazole and phenoxy pyridine scaffolds.

Scaffold	R

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