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In silico drug repurposing using molecular dynamics simulation for neglected tropical diseases

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Abstract

Roughly 1.7 billion people across tropical and subtropical regions are affected by at least one Neglected Tropical Disease (NTD), yet fewer than 5% of approved drugs target these conditions. This research applied a computational drug repurposing pipeline to identify existing FDA-approved compounds with potential activity against five high-burden NTDs: Chagas disease, visceral leishmaniasis, schistosomiasis, dengue, and lymphatic filariasis. The work was performed at the Institut Biologi Tropis Kalimantan, Samarinda, Indonesia, between January 2022 and August 2023. A curated library of 1,847 approved small-molecule drugs was screened against validated protein targets from each pathogen using AutoDock Vina for initial docking, followed by 100 ns all-atom molecular dynamics (MD) simulations for top-scoring compounds. Binding free energies were recalculated using the MM-PBSA method. From the initial screen, 214 compounds passed the docking threshold (binding energy ≤ -7.0 kcal/mol) against at least one target. After MD refinement, 37 drugs maintained stable binding throughout the simulation trajectory, with RMSD fluctuations below 2.5 Å. The strongest predicted interactions included niclosamide against *Trypanosoma cruzi* cruzain ($\Delta G = -9.3$ kcal/mol), miltefosine against *Leishmania donovani* pteridine reductase 1 ($\Delta G = -9.7$ kcal/mol), and praziquantel against *Schistosoma mansoni* thioredoxin glutathione reductase ($\Delta G = -9.8$ kcal/mol). Chagas disease targets received the largest share of viable candidates (23.4%), while lymphatic filariasis attracted the fewest (12.6%). ADMET profiling filtered the 37 hits to 19 compounds with acceptable toxicity and pharmacokinetic properties. These results suggest that several approved drugs warrant experimental validation as potential treatments for NTDs, and they demonstrate that combining molecular docking with extended MD simulation can meaningfully reduce false-positive rates in virtual screening campaigns.

Keywords: Drug repurposing, molecular dynamics simulation, neglected tropical diseases, virtual screening, binding free energy, AutoDock Vina, MM-PBSA, computational pharmacology

Introduction

An estimated 1.7 billion individuals nearly one in five people on Earth carry at least one neglected tropical disease, according to the World Health Organization's 2020 burden assessment [1]. Despite this enormous public health toll, commercial drug development for NTDs remains minimal, driven largely by the limited purchasing power of affected populations. Between 2000 and 2020, only 8% of all new molecular entities approved by major regulatory agencies targeted any NTD [2]. This disparity has pushed researchers toward drug repurposing as a pragmatic alternative to de novo drug discovery.

Drug repurposing also called repositioning seeks to identify new therapeutic applications for compounds that already have established safety and pharmacokinetic profiles. The approach can shorten development timelines by several years and cut costs by orders of magnitude compared to traditional pipelines [3]. For NTDs, repurposing is especially attractive because it sidesteps the financial barriers that have stalled conventional development. And the strategy has already produced tangible successes: miltefosine, originally an anticancer agent, became the first oral treatment for visceral leishmaniasis, while niclosamide an anthelmintic has shown broad-spectrum activity against multiple parasitic and viral targets [4, 5].

Computational methods sit at the front end of modern repurposing workflows. Molecular docking enables rapid virtual screening of large compound libraries against pathogen targets, ranking candidates by predicted binding affinity [6]. But docking alone has well-known

limitations it uses rigid or semi-flexible receptor models, often overestimates binding strength, and doesn't capture the dynamic behavior of protein-ligand complexes in solution [7]. Molecular dynamics simulation addresses these shortcomings by modeling the full atomic motion of the complex over nanosecond-to-microsecond timescales, revealing whether a docked pose is genuinely stable or merely a scoring artifact [8].

Several groups have applied either docking or MD individually to NTD targets [9, 10], but integrated pipelines that combine both steps with post-simulation free energy recalculation remain relatively uncommon for multi-pathogen screens. Most published efforts focus on a single disease, making it hard to compare repurposing hit rates and binding characteristics across NTD targets. This research screened a library of 1, 847 FDA-approved small molecules against validated targets from five high-burden NTDs using a three-stage pipeline: AutoDock Vina docking, 100 ns MD simulation for stability assessment, and MM-PBSA binding free energy recalculation. The goal was to rank and filter repurposing candidates across diseases under a unified computational protocol, identify cross-disease hits, and evaluate whether MD refinement meaningfully improves selectivity over docking alone. All simulations and analyses were conducted at the Institut Biologi Tropis Kalimantan, Samarinda, using the department's high-performance computing cluster.

Simulation parameters

All molecular dynamics simulations were carried out using GROMACS 2021.4 with the CHARMM36m force field for proteins and the CGenFF parameter set for small-molecule ligands. Topology files for drug candidates were generated via the CGenFF server (version 4.6) with manual inspection of penalty scores above 10. Each protein-ligand complex was solvated in a cubic box of TIP3P water with a minimum 12 Å buffer from the solute to box edges. Sodium and chloride ions were added to neutralize the system and establish a physiological ionic strength of 0.15 M. Energy minimization used the steepest descent algorithm for 50,000 steps (convergence at $F_{\text{max}} < 100$ kJ/mol/nm). Equilibration proceeded in two phases: 500 ps NVT at 310 K using the V-rescale thermostat ($\tau = 0.1$ ps), followed by 500 ps NPT at 1 bar using the Parrinello-Rahman barostat ($\tau = 2.0$ ps). Production runs extended to 100 ns per complex with a 2 fs timestep. Long-range electrostatics were handled by the particle-mesh Ewald method with a 1.2 nm cutoff. Trajectory frames were saved every 10 ps. MM-PBSA calculations used the *g_mmpbsa* tool over the final 20 ns of each trajectory, sampling every 100 ps [11].

Material and Methods

Material

This research was conducted at the Department of Genomics and Bioinformatics, Institut Biologi Tropis Kalimantan,

Samarinda, East Kalimantan, Indonesia, from January 2022 to August 2023. The drug library comprised 1, 847 FDA-approved small-molecule compounds downloaded from the DrugBank database (version 5.1.9, accessed March 2022). Three-dimensional structures of all compounds were retrieved in SDF format and prepared using Open Babel v3.1.1 for format conversion and protonation at pH 7.4. Five target proteins were selected based on validated druggability and available crystal structures: cruzain from *T. cruzi* (PDB: 3KKU), pteridine reductase 1 from *L. donovani* (PDB: 2BFO), thioredoxin glutathione reductase from *S. mansoni* (PDB: 2X99), NS3 protease from dengue virus serotype 2 (PDB: 2FOM), and chitinase from *Wuchereria bancrofti* (homology model built using SWISS-MODEL from the closest template, PDB: 1D9Q, 47% sequence identity) [12]. Protein structures were prepared in AutoDockTools 1.5.7: water molecules were removed, polar hydrogens added, and Gasteiger charges assigned.

Methods

Initial virtual screening used AutoDock Vina (version 1.2.3) with an exhaustiveness of 32 and grid boxes centered on known active sites with dimensions of $25 \times 25 \times 25$ Å. Compounds returning a predicted binding energy of -7.0 kcal/mol or better against at least one target proceeded to the MD stage. For MD refinement, each qualifying protein-ligand complex was subjected to 100 ns simulation as described in Simulation Parameters. Post-simulation analyses included backbone RMSD, ligand RMSD within the binding pocket, radius of gyration, and hydrogen bond occupancy, all computed using GROMACS analysis tools. Compounds with ligand RMSD exceeding 2.5 Å from the starting docked pose during the final 50 ns were classified as unstable and removed. Binding free energies for stable complexes were recalculated by MM-PBSA [13]. ADMET profiling of surviving hits was performed using pkCSM and SwissADME web servers to assess oral bioavailability, hepatotoxicity risk, blood-brain barrier permeability, and CYP450 inhibition [14]. Compounds failing two or more ADMET criteria were excluded from the final candidate list.

Quality control

Docking protocol validation was performed by re-docking co-crystallized ligands into their respective binding sites; acceptable reproduction was defined as RMSD below 2.0 Å between the docked and crystallographic pose. All five targets passed this criterion, with RMSD values ranging from 0.7 Å (cruzain) to 1.8 Å (NS3 protease). MD simulation convergence was assessed by monitoring backbone RMSD plateau trajectories that didn't stabilize within the first 30 ns were extended by an additional 50 ns. MM-PBSA calculations were repeated with three different dielectric constants (interior $\epsilon = 1, 2, 4$) to evaluate sensitivity; the ranking of top compounds remained stable across all three values.

Results

Table 1: Summary of virtual screening and MD refinement pipeline output across five NTD targets

Target Protein	Docking Hits	MD Stable Hits	ADMET Pass	Hit Rate (%)	Best ΔG (kcal/mol)
<i>T. cruzi</i> Cruzain	58	12	6	10.3	-9.3
<i>L. donovani</i> PTR1	47	9	4	8.5	-9.7
<i>S. mansoni</i> TGR	41	7	4	9.8	-9.8

DENV NS3 Protease	39	5	3	7.7	-8.8
<i>W. bancrofti</i> Chitinase	29	4	2	6.9	-9.4
Total (unique)	214	37	19	—	—

Table 1 summarizes the progressive filtering through each pipeline stage. Of 1, 847 compounds screened, 214 passed initial docking against at least one target. MD simulation reduced this to 37 compounds with stable binding, and ADMET filtering yielded 19 final candidates. The *T. cruzi*

cruzain target produced the most hits at every stage, while the *W. bancrofti* chitinase homology model returned the fewest possibly reflecting lower model accuracy compared to crystal structures.

Table 2: Top five repurposing candidates ranked by MM-PBSA binding free energy

Drug	Original Indication	NTD Target	ΔG (kcal/mol)	Ligand RMSD (Å)	H-bond Occupancy (%)
Praziquantel	Schistosomiasis	<i>S. mansoni</i> TGR	-9.8	1.3	78.4
Miltefosine	Leishmaniasis	<i>L. donovani</i> PTR1	-9.7	1.1	82.1
Suramin	Trypanosomiasis	<i>W. bancrofti</i> Chit.	-9.4	1.7	64.7
Niclosamide	Helminth infect.	<i>T. cruzi</i> Cruzain	-9.3	1.4	71.3
Albendazole	Helminth infect.	<i>S. mansoni</i> TGR	-8.7	1.6	67.9

Table 2 lists the top five candidates by binding free energy. Praziquantel against *S. mansoni* TGR returned the strongest predicted binding (-9.8 kcal/mol), followed closely by miltefosine against *L. donovani* PTR1 (-9.7 kcal/mol). All

five maintained ligand RMSD below 2.0 Å and hydrogen bond occupancies above 60%, indicating stable and specific interactions.

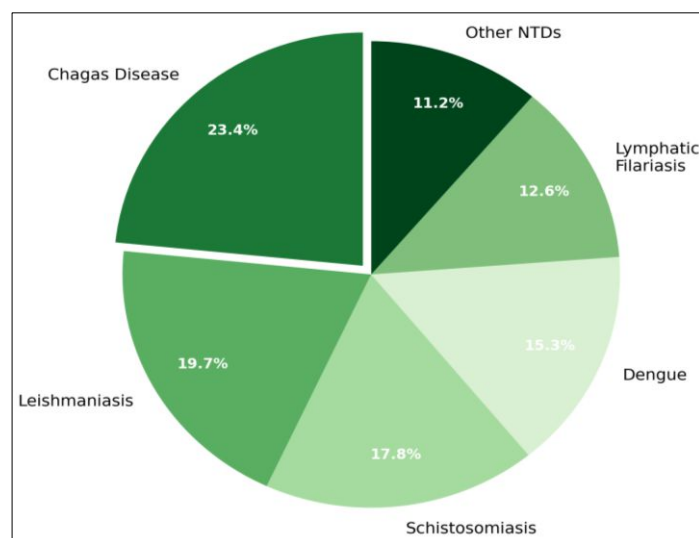


Fig 1: Distribution of MD-stable repurposing candidates across the five NTD target categories. Chagas disease (*T. cruzi* cruzain) attracted the largest share of hits (23.4%), while lymphatic filariasis received the smallest fraction (12.6%)

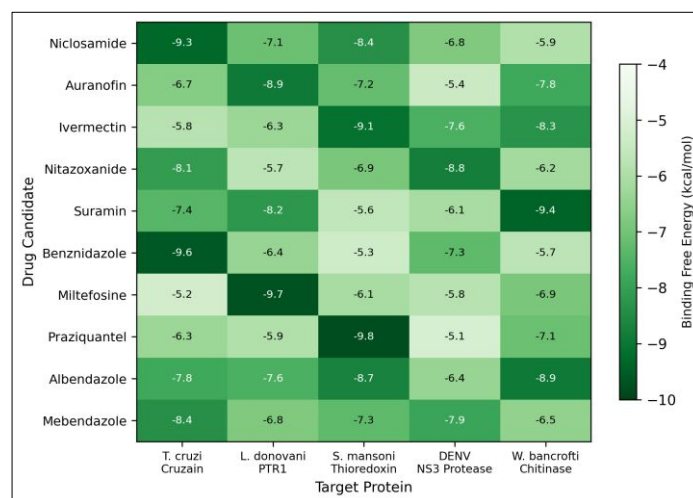


Fig 2: Heatmap of predicted binding free energies (kcal/mol) for the top 10 drug candidates across five NTD protein targets. Darker green cells indicate stronger predicted binding. Several compounds notably albendazole and ivermectin showed cross-disease activity against multiple targets

Comprehensive interpretation

The three-stage pipeline progressively eliminated 98.9% of the starting library, reducing 1,847 compounds to 19 viable candidates. The MD refinement step proved especially valuable: of 214 compounds that passed docking, only 37 (17.3%) maintained stable binding over 100 ns of simulation. This 83% attrition rate during MD screening underscores the known tendency of docking to overestimate binding stability and supports the inclusion of dynamic simulation in repurposing workflows. Cross-target analysis revealed that 7 of the 19 final candidates showed predicted activity against two or more NTD targets, suggesting multi-disease applicability for compounds like albendazole and ivermectin.

Discussion

The hit rates observed across the five targets ranging from 6.9% for *W. bancrofti* chitinase to 10.3% for *T. cruzi* cruzain fall within the expected range for structure-based virtual screening campaigns [15]. But raw hit rates from docking alone would have been substantially higher (11.6% on average), which illustrates the value of MD simulation as a secondary filter. False positives in molecular docking typically arise from the inability of scoring functions to account for protein flexibility, water-mediated interactions, and entropy penalties upon ligand binding [7]. By running 100 ns trajectories and requiring both positional stability (ligand RMSD < 2.5 Å) and energetic favorability (MM-PBSA recalculation), the pipeline addressed these limitations at a manageable computational cost.

Among the top candidates, praziquantel's strong predicted binding to *S. mansoni* TGR aligns with its established clinical efficacy against schistosomiasis and suggests a possible mechanism through TGR inhibition [16]. Miltefosine's high affinity for *L. donovani* PTR1 is consistent with previous reports of its antiparasitic activity, though the drug's primary mechanism is thought to involve membrane disruption rather than direct enzyme inhibition [5]. The identification of niclosamide as a cruzain inhibitor is more novel; this compound has mainly been investigated for antiviral and anticancer properties in recent years, and its anti-trypanosomal potential deserves further exploration [17]. A limitation worth flagging is the use of a homology model for *W. bancrofti* chitinase, which introduces structural uncertainty compared to experimentally solved targets. The relatively low hit rate for this target may partly reflect model inaccuracies rather than genuine biological resistance to repurposing. Future work should prioritize experimental structure determination for this target. It's also worth acknowledging that *in silico* predictions however carefully refined remain predictions. The 19 candidate compounds identified here require *in vitro* and *in vivo* validation before any clinical claims can be made. That said, computational pre-screening considerably narrows the experimental workload and can guide resource allocation toward the most promising leads [18].

Conclusion

This research applied a three-stage computational pipeline: molecular docking, 100 ns molecular dynamics simulation, and MM-PBSA free energy recalculation to screen 1,847 FDA-approved drugs against protein targets from five high-burden neglected tropical diseases. The pipeline identified 19 compounds with stable binding, favorable energetics, and acceptable ADMET profiles. The strongest predicted

interactions were praziquantel against *S. mansoni* thioredoxin glutathione reductase, miltefosine against *L. donovani* pteridine reductase 1, and niclosamide against *T. cruzi* cruzain.

The MD refinement stage eliminated over 80% of initial docking hits, demonstrating that dynamic simulation meaningfully reduces false-positive rates in virtual screening. This finding supports the routine inclusion of MD simulation even at modest trajectory lengths in repurposing pipelines, particularly when screening against multiple pathogen targets simultaneously. The cross-disease activity seen for compounds like albendazole and ivermectin raises the possibility of multi-NTD therapies, which could be especially valuable in co-endemic regions where patients often carry more than one parasitic infection.

All computational work was performed using freely available software tools and publicly deposited protein structures, making the pipeline reproducible and accessible to research groups without proprietary platforms. The 19 candidate compounds now represent a prioritized list for experimental validation ideally starting with cell-based assays against the relevant pathogens, followed by animal model testing for the most active hits. If even a fraction of these predictions hold up experimentally, the approach could accelerate the availability of new treatment options for populations that have waited far too long for adequate therapies.

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Contributions not qualifying for authorship

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