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Exploring therapeutic potential of repurposed drugs in chagas disease: An integrated network pharmacology and molecular docking approach

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Chagas disease, a neglected tropical illness caused by *Trypanosoma cruzi* characterized by severe inflammatory response and lethal cardiac complications, making traditional chemo-therapeutics inefficient. Through advanced molecular docking techniques and Network pharmacological approach, we screened 20 FDA approved orphan drugs against Chagas disease to identify their possible mechanism of action. Further these targets were validated by reviewing series of case studies conducted in patients with Chagas disease. Initially the 20 FDA approved drugs were subjected to molecular docking against *Trypanosoma cruzi* glucokinase. Thirteen Promising drugs with strong binding affinity were chosen for pharmacogenomic profiling through network pharmacology analysis to elucidate complex drug-target-disease relationships, potential therapeutic mechanisms and drug re-purposing opportunities. Our integrated methodology revealed that drugs like Belumosudil, Quizartinib, Vomorolone, Voydeya and Zanubritinib are directly involved in pathways related to Chagas disease. Further docking analysis was conducted between these drugs and their respective highly interacting gene. A detailed case report analysis confirmed their role in pathogenesis of *T. cruzi* infection. Other 8 drugs are found to be indirectly effective by targeting inflammatory pathways of disease progression. This research presents a multifaceted investigation integrating molecular docking, network pharmacology, and clinical case analysis to explore novel therapeutic strategies for managing the disease.

Keywords: Bio-informatics, Chagas disease, Drug re-purposing, *In silico* drug discovery, Molecular targets, *Trypanosoma cruzi*

Chagas disease is a protozoan disease caused by parasite *Trypanosoma cruzi*, a blood-sucking triatomines, affecting host immune response. The disease gets transmitted through blood transfusion, congenital transmission, contaminated meals, vertical transmission and feces of infected triatomine bugs[1, 2]. Once entering the host, the parasite invades primarily immune cells like macrophages and fibroblast and uses host cell machinery to multiply within host resulting in lysis of affected cells and release of new trypomastigotes in host blood[3, 4]. The progression of disease triggers the host's immune response and releases immune cells and pro-inflammatory cytokines like TNF-α, IL-6 and cause collateral tissue damage[5]. Myocarditis is the common incident occurring in this phase of disease caused by infiltration of immune cells and necrosis in cardiac myocytes. When the disease is not treated, the parasite resides in cardiac and other smooth muscle tissues, leading to severe cardiac and gastrointestinal illness. In many cases, persistent of parasite in host for long time leads to chronic phase of disease, which is characterized by reduced immune response that allows the parasite to evade destruction[6]. The chronic persistence of the disease can trigger an autoimmune reaction, affecting the host's own tissues and leading to fibrosis. The presence of molecular mimicry between *T. cruzi* antigen and host tissue was found to be the factor that contributes to autoimmune response. The chronic phase of the disease has highest rate of morbidity due to cardiac complications like chronic myocarditis, systolic dysfunction, arrhythmia and fibrosis. Gastrointestinal complications like severe constipation, dysphagia, megacolon, megaesophagus caused by enteric neuron damage, has also reported to increase morbidity in chagas disease[7]. Overall, the host immune system which is responsible for controlling infection causes tissue damage and adds on complication to ongoing inflammatory process[8]. Being untreated, this chronic life-threatening infection can persist throughout life. Combined with parasite persistence, this disease results in wide array of clinical manifestations that pose challenges for effective treatment and management.

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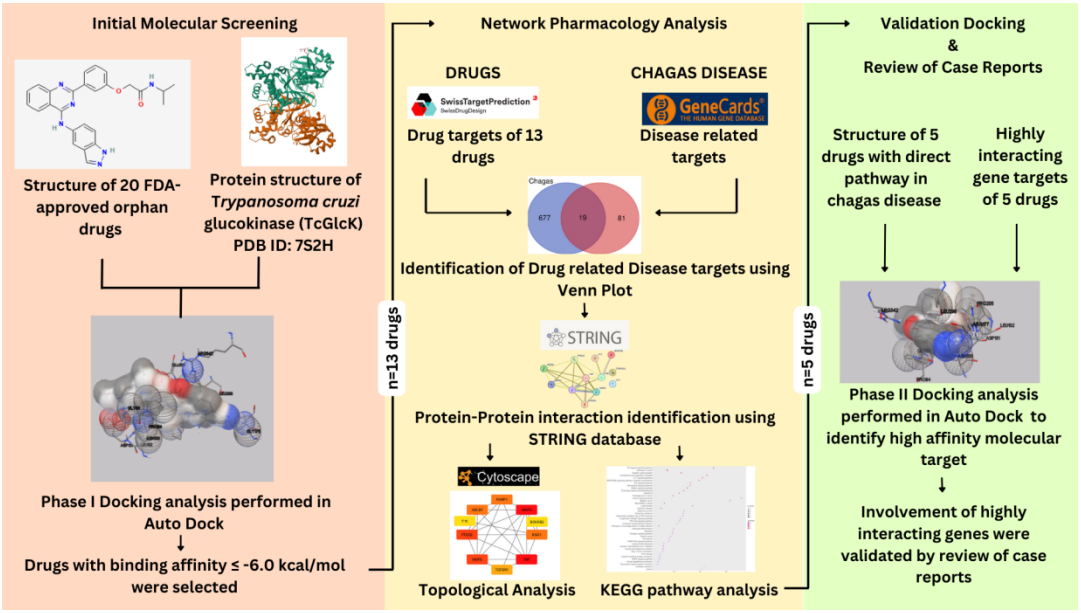

Graphical abstract

Challenges faced during diagnosing the disease with available diagnostic methods and lack of proper treatment strategy in a resource-limiting setting prolongs the turnaround time and mortality rate[9-12]. This emphasizes the need for new diagnostic tools and treatment regimen to identify and treat the disease at the earliest possible. Current treatment strategies involve anti-parasitic medications like benznidazole and nifurtimox. But these medications are effective only in acute phase of the disease and offer limited efficacy in chronic cases[13]. The aim of WHO 2030 road-map is to eliminate the disease as it concerns public health and focus on timely diagnosis to enhance the successful treatment outcome. Being a Neglected Tropical Disease (NTD) by the World Health Organization, this disease needs urgent attention and innovated treatment to improve the life-threatening complications of the disease[14,15]. We performed molecular docking analysis of totally 20 FDA approved orphan drugs from the year 2023 to 2024 against *Trypanosoma cruzi* glucokinase (TcGlcK) protein which is responsible for parasite replication inside the host. Identified candidates with strong binding affinity were subjected to Network pharmacology screening to explore their molecular mechanism. The knowledge from various databases is used to identify the mechanism of action of drugs and genes of disease which interacts with them. Finally a detailed case report provides real-world validation of our molecular findings and compiles the clinical manifestations of the disease.

Materials and Methods

Study design overview

This study utilized a systematic computational drug repurposing method that included molecular docking and network pharmacology to discover prospective therapeutic options from FDA-approved orphan medicines for the treatment of Chagas disease. The methodology (Fig. 1) comprised three distinct phases: initial molecular screening, network pharmacology analysis, and targeted validation docking using AutoDock Vina (version 4.2.6).

Phase-1: Initial molecular docking screening

Protein preparation

The atomic coordinates of unbound, wild type natural form of enzyme *Trypanosoma cruzi* glucokinase (TcGlcK) with accession code 7S2H was retrieved from Protein Data Bank. Protein preparation was performed using PyMOL (version 2.5.0) and AutoDockTools (version 1.5.7). Water molecules and hetero atoms removed within 5 Å of binding site and structure is Optimized at pH 7.4. Finally, the structure is converted into PDBQT format.

Ligand preparation

An extensive data set collection of 20 FDA-approved orphan drugs was compiled from the FDA Orphan Drug Database. Drug structures were acquired in SDF format from the PubChem database. All structures were standardized using Open Babel (version 3.1.1) set with optimization parameters: pH

Fig. 1 — Workflow for screening and identification of potential orphan drugs for Chagas disease treatment

normalization to 7.4; energy minimization employing the UFF force field of 500 minimization steps and removal of duplicate conformers using RMSD threshold of 0.5 Å.

Docking analysis

Docking analysis was performed using Auto Dock software version 4.2.6 to identify its binding affinity and visualize the binding sites. The software uses empirical scoring function that contains van der Waals, hydrogen bonding, electrostatic, and desolvation energies for scoring. The lower the binding energy, the better the docking score, so binding affinity threshold was set ≤ -6.0 kcal/mol as primary cutoff and pose clustering threshold was set to RMSD-based clustering with 2.0 Å threshold. Compounds meeting the binding affinity criteria were advanced to network pharmacology analysis.

Network pharmacological analysis

Prediction of drug targets

SwissTargetPrediction database was used to screen drug related targets. It predicts the target of given molecule by identifying proteins that are similar to known ligands, based on the principle that similar bio-active molecules are likely to share similar targets[16]. The smiles of the selected drug molecules were obtained from PubChem database and uploaded in SwissTargetPrediction for target fishing. Targets from Homo-sapiens species are filtered in order to increase the credibility.

Prediction of disease targets

GeneCards database, a collection of wide variety of human genes, disease, variants, proteins, cells and biological pathway was used to collect targets related to Chagas disease[17]. The potential targets of the drug relevant to targets involved in Chagas disease were identified using Bio informatics& Evolutionary genomics web tool.

Network analysis

STRING database, a database known for predicting protein-protein interaction including physical and functional association between molecules, with about 67'592'464 proteins from 14'094 organisms was used to obtain protein-protein interaction network[18]. The potential targets found form Bioinformatics & Evolutionary genomics web tool was uploaded in STRING database and particularly targets from Homo-sapiens species were selected to obtain confidence score. Cytoscape, an open-source software was used to visualize molecular interaction and Cyto-Hubba plug-in feature of the software was used to calculate the topological parameters of the network. Top 10% of nodes with degree and betweenness centrality were identified as hub genes.

Gene ontology enrichment analysis

Gene Ontology analysis was performed to reveal the potential biological function of the selected drugs with relation to their treatment in Chagas disease. DAVID database provides a comprehensive set of functional annotation tools to understand biological meaning of large set of genes[19]. The targets of our selected drugs were uploaded in DAVID database to analyse their functional enrichment and the results were filtered with a threshold value of FDR< 0.05 and Adjusted p-value < 0.01.

KEGG pathway analysis

DAVID database is used to obtain the KEGG pathway along with gene ID, P value and fold enrichment. A limit was set to Benjamini-corrected p-value < 0.05 and fold enrichment requirement as minimum 2.0-fold, for considering significance.

The obtained genes are uploaded in Shiny GO 0.77 and Homo-sapiens species was selected. Finally, the KEGG pathway enrichment graph was obtained.

Phase 3: Targeted validation docking: Docking analysis and Target validation of highly interacting genes

The drugs which target Chagas disease directly are identified through KEGG analysis and those drugs were subjected to docking with their highly interacting gene to elucidate their possible mechanism of action. Molecular docking was carried out using Auto Dock software. Finally, the involvement of core target genes of drug in chagas disease were validated using review of case studies conducted in patients.

Results

Molecular docking

The molecular docking analysis of 20 FDA-approved orphan drugs against the Trypanosoma cruzi glucokinase (TcGlcK) protein (PDB accession code 7S2H) associated with Chagas disease revealed good binding interactions (Table 1). Seven out of 20 drugs namely Filspari, Givinostat, Hepzatokit, Jaypirca, Leniolisib, Tovorafenib and Trofinetide showed weak binding affinity ranging from -3.02 kcal/mol to -6.97 kcal/mol. Remaining 13 drugs showed strong binding affinity ranging from -7.22 kcal/mol to -9.45 kcal/mol. These 13 drugs were further subjected to Network analysis.

Table 1 — Binding affinity of 20 Orphan drugs against *Trypanosoma cruzi* glucokinase (TcGlcK) protein

S. No	Name of the Drug	Docking Score with 7S2H protein
1.	Alectinib	-9.45 kcal/mol
2.	Ayvakit	-8.84 kcal/mol
3.	Belumosudil	-8.68 kcal/mol
4.	CCX168	-9.11 kcal/mol
5.	Filspari	-5.06 kcal/mol
6.	Givinostat	-6.97 kcal/mol
7.	Hepzatokit	-4.43 kcal/mol
8.	Jaypirca	-5.74 kcal/mol
9.	Leniolisib	-6.07 kcal/mol
10.	Mavorixafor	-7.60 kcal/mol
11.	Momelotinib	-8.43 kcal/mol
12.	Palovarotene	-7.75 kcal/mol
13.	Quizartinib	-8.49 kcal/mol
14.	Resmetirom	-7.83 kcal/mol
15.	Ruxolitinib	-7.22 kcal/mol
16.	Tovorafenib	-5.50 kcal/mol
17.	Trofinetide	-3.02 kcal/mol
18.	Vamorolone	-7.38 kcal/mol
19.	Voydeya	-8.49 kcal/mol
20.	Zanubritinib	-7.66 kcal/mol

Target fishing

The targets of selected drugs with strong binding affinity were obtained from Swiss Target Prediction database using the smiles of the compounds obtained from PubChem database. The search term “chagas disease” was used as key word in GeneCards database to obtain relevant targets of disease. Total 696 targets related to disease were obtained from GeneCards database. The targets of selected drug candidates and targets of Chagas disease were compared individually to obtain the number of disease-related targets of individual drug (Table 2).

Disease- related drug target network

The obtained disease-related drug targets were uploaded in STRING database to obtain the protein-protein interaction network (Figs. 2-6). The resulting “tsv” file was visualized in cytoscape to perform network analysis. The cytohubba plug-in of cytoscape software was used to filter the highly interactive gene in the whole network. These targets have been determined to possess a greater degree value and could play a significant role in management of the disease.

Enrichment analysis

KEGG analysis

KEGG analysis done through ShinyGO showed that 5 drugs namely Belumosudil, Quizartinib, Vomorolone, Voydeya, Zanubritinib has significant number of genes that directly target chagas disease (Figs. 7-11), and others have considerable number of

Table 2 — Number of disease related targets present in selected drugs

S. No	Compound Name	Number of Disease Related Target
1.	Acetanib	19
2.	Belumosudil	12
3.	Ayvakit	11
4.	CCX168	09
5.	Mavorixafor	04
6.	Momelotinib	12
7.	Palovarotene	03
8.	Quizartinib	11
9.	Resmetirom	16
10.	Ruxolitinib	05
11.	Vamorolone	11
12.	Voydeya	12
13.	Zanubritinib	12

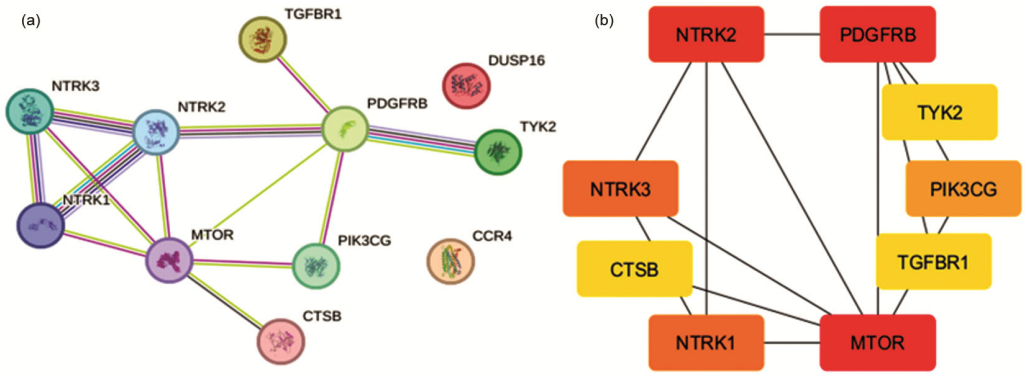

Fig. 2 — (a) Protein- Protein interaction; (b) and highly interacting gene network of Belumosudil

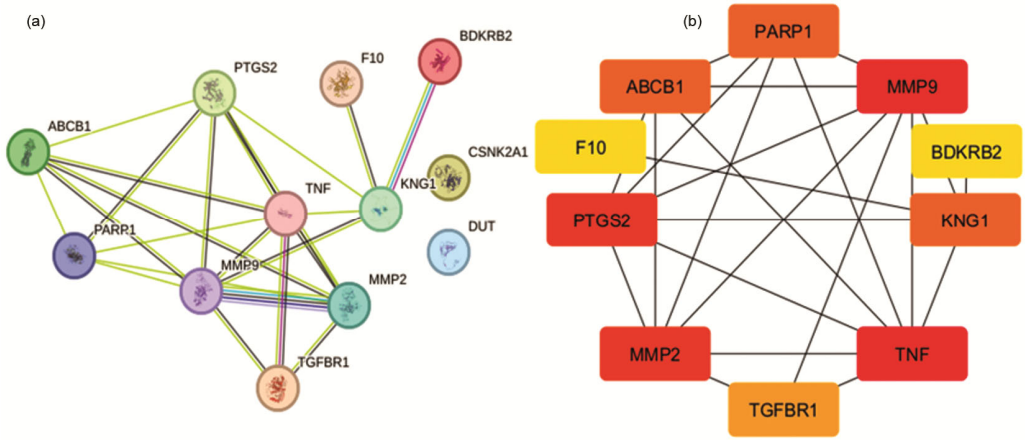

Fig. 3 — (a) Protein- Protein interaction; (b) and highly interacting gene network of Quizartinib

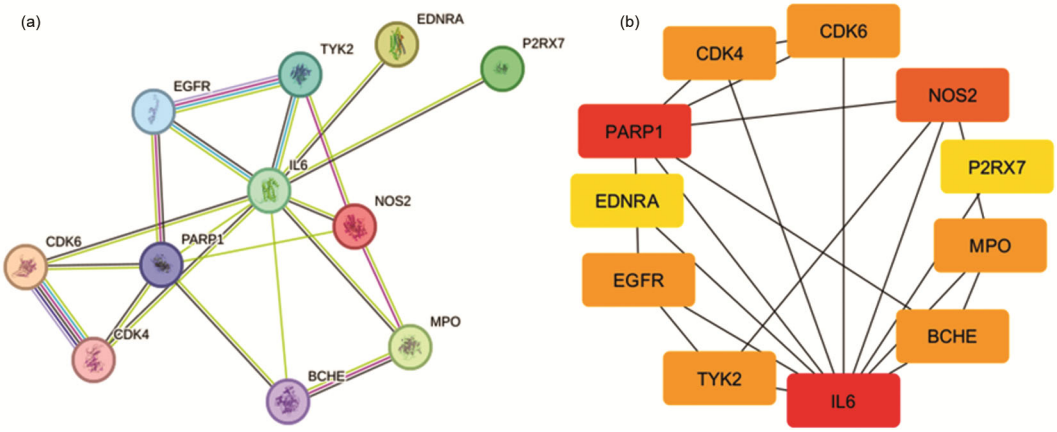

Fig. 4 — (a) Protein- Protein interaction; (b) and highly interacting gene network of Vomorolone

genes that target inflammation and pathways of disease progression. Other drugs also have significant amount of gene involved in influencing inflammatory pathways like PI3k-Akt signaling pathway, apoptosis, NF-Kappa B signaling pathway, IL-17 signaling pathway, TNF signaling pathway, RAS signaling pathway, TH17 cell differentiation, inflammatory mediator region of TRP channels. The molecular pathways of the drugs which indirectly target chagas disease are listed (Table 3).

Molecular docking analysis of Promising drug candidates with Highly interacting gene

The drugs that have genes which directly target chagas disease namely Belumosudil, Quizartinib, Vomorolone, Voydeya, Zanubritinib

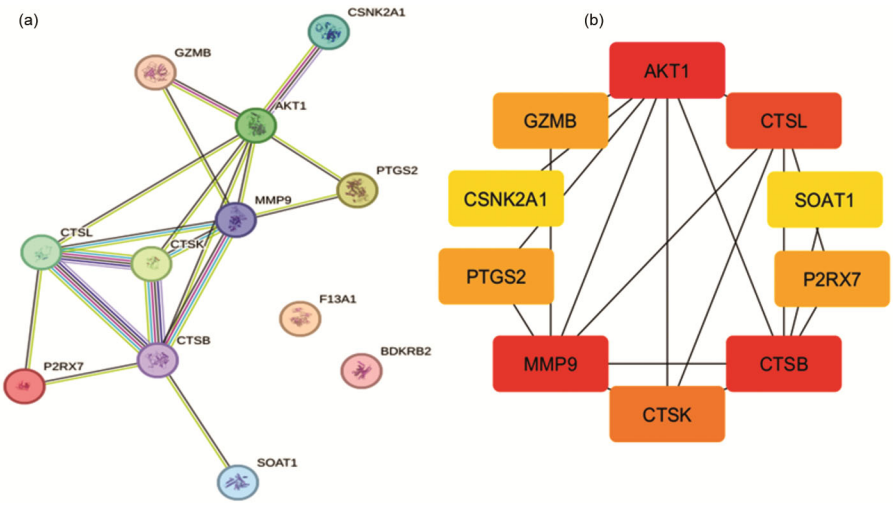

Fig. 5 — (a) Protein-Protein interaction; (b) and highly interacting gene network of Voydeya

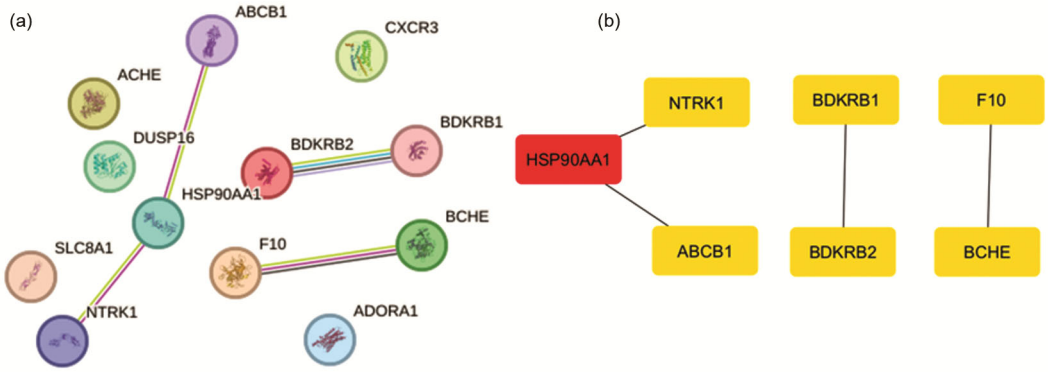

Fig. 6 — (a) Protein-Protein interaction; (b) and highly interacting gene network of Zanubritinib

were individually docked with their respective core gene hub to identify their affinity. From our docking analysis we found that Belumosudil shows lowest score with ABCB1 and highest score with MMP-2. Quizartinib shows lowest score with PDGFRB and highest score with CTSB. Vomorolone has lowest score with CDK4 and highest score with BCHE. Voydeya has lowest binding score with P2KX7 and highest score with MMP-9. Zanubritinib shows lowest score with BDKRB2 and highest score with HSP90AA1. The docking score of individual drugs with their respective core gene hub are tabulated (Table 4).

Clinical evidences

The role of individual gene in progression of Chagas disease is proved by series of case report and pre-clinical reports analysis. Pissetti CW conducted a study in municipality of Agua comprida, Brazil to analyse the role of TNF-alpha in chagas disease patients by conducting series of evaluation test like passive hem-agglutination, enzyme- linked immunosorbent assay, direct immunoflurescence. Clinical examination like electrocardiography, chest, esophagus and colon X-ray were done and identified that among 300 individuals participated in the study, 168 were found to be seropositive for *T. cruzi*, and among them 55.46 % were classified with cardiac form (CARD) and 44.54% with indeterminate form of Chagas disease. From the test results and clinical examinations, they concluded that seropositive individuals with indeterminate form (IND) of disease exhibited higher levels of TNF-alpha. They also found out that TNF-alpha has dual role in Chagas disease by controlling parasite growth in one end and also promoting tissue damage on the other. Their findings on association of TNF-alpha polymorphisms with disease revealed that individuals with TNF-238A allele produce higher levels of TNF-alpha. However they suggest that evaluating the amount of cells that

Fig. 7 — KEGG pathway enrichment analysis of drug-Belumosudil. Pathway names on Y-axis and fold enrichment on X-axis. Color gradient from red to yellow represents -log10 (FDR), with red indicating higher statistical significance and yellow indicating lower significance. Bubble/dot size indicates the number of genes involved in each pathway

Fig. 8 — KEGG analysis of drug-Qizartinib. Pathway names on Y-axis and fold enrichment on X-axis. Color gradient from red to yellow represents -log10 (FDR), with red indicating higher statistical significance and yellow indicating lower significance. Bubble/dot size indicates the number of genes involved in each pathway

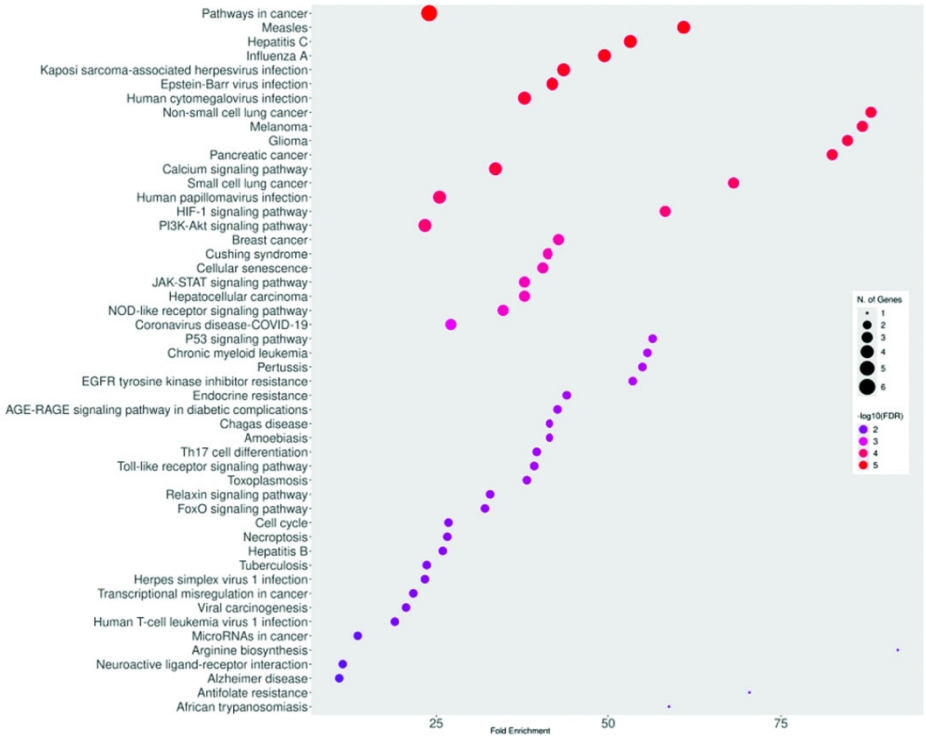

Fig. 9 — KEGG analysis of drug-Vomorolone. Pathway names on Y-axis and fold enrichment on X-axis. Color gradient from red to yellow represents -log10 (FDR), with red indicating higher statistical significance and yellow indicating lower significance. Bubble/dot size indicates the number of genes involved in each pathway

Fig. 10 — KEGG analysis of drug-Voydeya. Pathway names on Y-axis and fold enrichment on X-axis. Color gradient from red to yellow represents -log10 (FDR), with red indicating higher statistical significance and yellow indicating lower significance. Bubble/dot size indicates the number of genes involved in each pathway

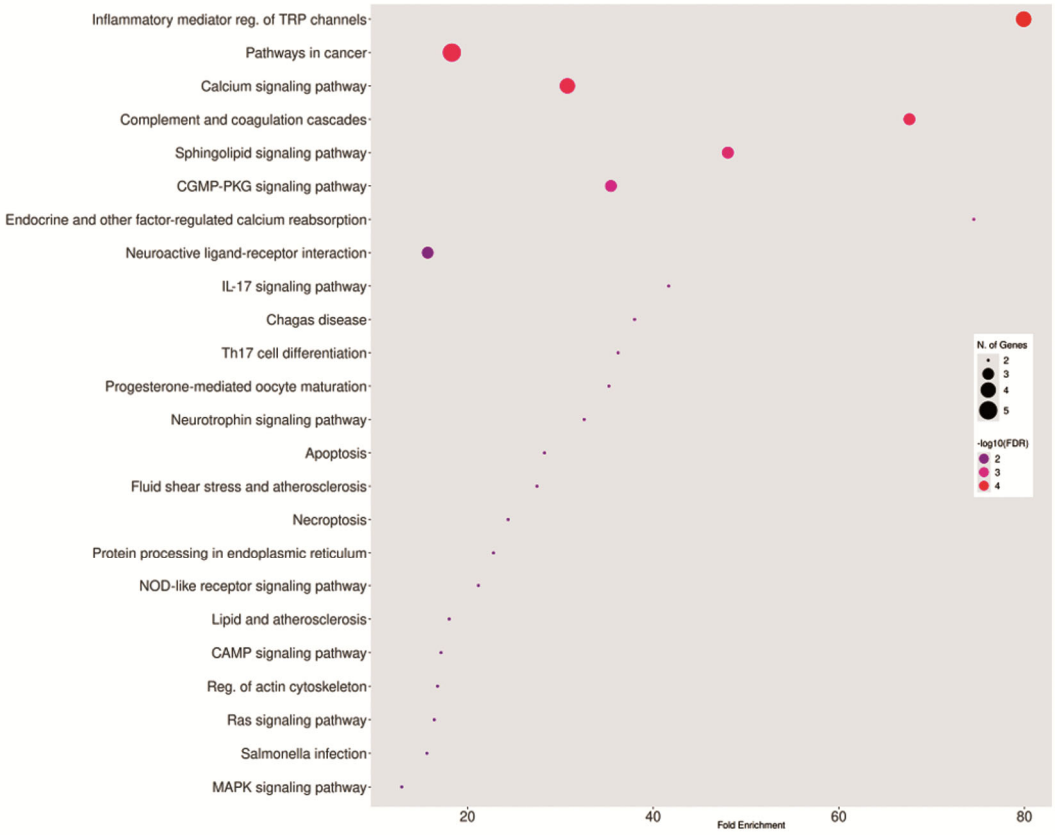

Fig. 11 — KEGG analysis of drug- Zanubritinib. Pathway names on Y-axis and fold enrichment on X-axis. Color gradient from red to yellow represents -log10 (FDR), with red indicating higher statistical significance and yellow indicating lower significance. Bubble/dot size indicates the number of genes involved in each pathway

Table 3 — Drugs and their molecular pathway that indirectly target chagas disease

S. No	Drugs	Pathway
1.	Alectinib	PI3k-Akt pathway, Chemokine signaling, EGFR tyrosine kinase inhibitor resistance, MAPK signaling, JAK-STAT pathway
2.	Ayvakit	NF-κB signaling, JAK-STAT pathway, MAPK signaling, EGFR tyrosine kinase inhibitor resistance, PI3k-Akt pathway, Toll-like receptor signaling, MTOR signaling
3.	CCX168	PI3k-Akt pathway, Ras signaling, JAK-STAT pathway
4.	Mavorixafor	PI3k-Akt pathway, EGFR tyrosine kinase inhibitor resistance, ErbB signaling, Th-17 differentiation, MTOR signaling
5.	Momelotinib	IL-17 signaling, TNF signaling, JAK-STAT pathway, EGFR tyrosine kinase inhibitor resistance, MAPK signaling, Th-17 differentiation, PI3k-Akt pathway, Ras signaling
6.	Palovarotene	JAK-STAT pathway, EGFR tyrosine kinase inhibitor resistance, Toll-like receptor signaling, Th-17 differentiation, Chemokine signaling
7.	Resmetirom	PI3k-Akt pathway, MAPK signaling, EGFR tyrosine kinase inhibitor resistance, ErbB signaling, IL-17 signaling, TNF signaling
8.	Ruxolitinib	Ras signaling, MAPK signaling, PI3k-Akt pathway, Th-1, Th-2 differentiation, IL-17 signaling, Inflammatory mediator regulation of TRP channels, Toll-like receptor signaling, NF-κB signaling, Th-17 differentiation, JAK-STAT pathway

produce cytokines and the amount of time that passes following contact with the parasite should be considered, in order to fully understand the role of TNF- alpha in Chagas disease[20].

Some series of case report findings shows that MMPs, especially MMP-2 and MMP9 in pathogenesis of cardiomyopathy caused by *T. cruzi* infection. The blood levels of MMP-2 and -9, their

Table 4 — Molecular docking results of potential drug candidates with highly interacting genes

Drug	PDB-ID	Gene name	Score (kcal/mol)	RMSD
Belumosudil	1B6C	TGFBR1	-6.4	38.11
	1L6J	MMP-9	-7.06	76.06
	1QIB	MMP-2	-12.04	71.94
	1TNF	TNF	-8.89	61.16
	1XKA	F10	-9.11	18.94
	4DQY	PARP1	-5.38	41.23
	5KIR	PTGS2	-8.65	46.07
	6JOD	BDKRB2	-5.86	49.84
	6QEX	ABCB1	-5.08	284.26
Quizartinib	1E8Y	PIK3CG	-8.30	32.01
	1GMY	CTSB	-9.85	72.22
	3MJG	PDGFRB	-6.89	36.37
	4ATS	NTRK2	-9.64	25.33
	4F0I	NTRK1	-7.88	73.12
	4GVJ	TYK2	-9.21	15.68
Vamorolone	1ALU	IL-6	-7.88	25.60
	1BLX	CDK6	-7.71	68.57
	1CXP	MPO	-7.16	14.19
	1M17	EGFR	-7.55	55.39
	1NSI	NOS2	-7.08	83.17
	1P0Y	BCHE	-8.77	199.07
	2W9Z	CDK4	-5.81	51.38
	4DQY	PARP1	-6.09	103.78
Voydeya	4GVJ	TYK2	-7.78	11.69
	5GLH	EDNRA	-7.09	21.29
	1ATK	CTSK	-7.64	65.96
	1GMY	CTSB	-8.69	67.19
	1QIP	MMP-9	-10.02	65.98
	3CQW	AKT-1	-8.42	24.15
	5KIR	PTGS2	-6.82	27.59
	6U9V	P2KX7	-6.43	244.82
Zanubritinib	1P0I	BCHE	-10.13	168.79
	1UYF	HSP90AA1	-11.48	22.16
	1XKA	F10	-8.70	21.97
	4F0I	NTRK1	-7.32	47.36
	6JOD	BDKRB2	-6.82	77.36
	6QEX	ABCB1	-9.23	37.87

possible proteolytic activity, their primary origins within mono-nuclear cells and their potential to cause complications of heart failure in human Chagas disease were originally described by Fares RCG et al. in 2013. They discovered that in addition to mono-nuclear cells, CD4+ and CD8+ T lymphocytes as well as CD14+ monocycle can generate MMP-2 and MMP-9. From their findings, they speculate that cardiac form and indeterminate form of chagas disease in patients exist due to the role of MMPs in their body. Predominance of MMP-9 results in intense cardiac remodeling leading to development of cardiac form and MMP-2 predominance leads to development of indeterminate phase of chagas disease[21, 22]. Another prospective cohort study conducted for 17 years by Nayara in 2017 discovered that neutrophils express more MMPs than monocytes. This suggests that neutrophils are a significant source of MMPs in Chagas disease and may be linked to inflammation and the development of Chagas cardiomyopathy. According to the findings, the IND group produces a significant amount of regulatory molecules including MMP-2, TGF-β, and IL-10, which may have a modulator influence on inflammatory molecules like MMP-9, TNF-α, and IL-1β. In contrast, the CARD group had elevated levels of inflammatory mediators, including MMP-9, TNF-α, and IL-1β, which could be linked to the escalation of inflammation and the development of cardiac abnormalities[23].

Discussion

Chagas disease, caused by *T. cruzi*, is a severe protozoa infection with a severe inflammatory response and lethal cardiac complications, making traditional chemo-therapeutics inefficient[24, 25]. Being stated as a neglected tropical disease with an estimated 6-7 million affected people, the day by day increase in people at risk of infection demands a potent drug to treat its life-threatening complications. We carried out a integrated computational approach to identify promising drug candidate that can be suitable to treat this infection and its pathological complications. Initially molecular docking of orphan drugs approved by FDA between the period of 2023 to 2024 was performed against *Trypanosoma cruzi* glucokinase protein, which plays a major role in invasion of pathogen and progression of disease in host body. This was done to screen the 20 drugs and identify suitable drugs which can be effective in treating the pathogenic invasion. The docking scores of 20 FDA approved orphan drugs with apo-wtTcGlcK ranged from least being -3.02 kcal/mol for Trofinetide to highest being -9.45 kcal/mol for Alectinib. The drugs with strong binding affinity (threshold <6.0 kcal/mol) ranging from -7.22 kcal/mol to -9.45 kcal/mol were chosen for next stage of the research. Out of 20 drugs, 13 drugs showed strong binding score with *Trypanosoma cruzi* glucokinase protein. On analyzing their pharmacogenomic profiling through KEGG analysis drugs namely Belumosudil, Quizartinib, Vomorolone, Voydeya, Zanubritinib has significant amount of genes that directly target chagas

disease. Others have considerable amount of genes that target inflammation pathways of disease progression (Table 3).

Belumosudil showed high interaction with TNF and MMP-9. KEGG analysis revealed significant pathways in Chagas disease pathogenesis including NF-κB signaling, IL-17 signaling, and TNF signaling. Matrix metalloproteinases 2, 3, 8, and 9 are proven involved in myocardial fibrosis and tissue remodeling[26-28]. Targeting inflammatory mediators like TNF[20, 29, 30], IL-17, and NF-κB could prevent inflammation and apoptosis worsening cardiac myopathy. Other interacting genes (TGFBR1, PARP1, PTGS2, BDKRB2) showed good binding affinity (-12.04 to -5.38 kcal/mol) and are involved in inflammatory cascade, fibrosis regulation[31, 32], and cardiac apoptosis[33].

Quizartinib highly interacted with MTOR gene, implicated in *T. cruzi* survival within host cells. Significant target pathways include Chagas disease pathogenesis, MAPK signaling, and PI3k-Akt pathway, effectively disrupting *T. cruzi* survival and reducing parasitic load[34]. The drug also interacts with PIK3CG, TYK2, PDGFRB, NTRK2, and CTSB, regulating inflammatory responses[33] and immune cell activation[35, 36]. Strong binding affinity (-7.88 to -9.85 kcal/mol) confirms potential reduction of disease complications.

Vomorolone interacted with IL-6 and showed involvement in PI3k-Akt pathway, JAK-STAT pathway, and TH-17 differentiation. IL-6 induces myeloid-derived suppressor cells recruitment and modulates cardiac infection response and regulation of NO production by controlling IL-1β[37, 38]. Docking scores (-5.81 to -8.77 kcal/mol) with network genes (CDK6, NOS2, P2RX7, MPO, EGFR) indicate potential action against inflammatory cytokines recruitment. By targeting TYK2, NOS2, and IL-6, the drug can suppress immune modulation and reduce neuroinflammation[29]. MPO generates ROS for pathogen killing but can cause tissue damage and myocardial injury[39-40]. Other genes like CDK6 involving in cell proliferation, EGFR in cell growth and survival, EDNRA in vasoconstriction, CDK4 in cell cycle has less significance in *T. cruzi* infection[41].

Voydeya interacts with CTSL, SOAT1, P2RX7, CTSB, CTSK, and shows high interaction with AKT1. Good binding scores (-6.43 to -10.02 kcal/mol) suggest modulation of immune-mediated response. KEGG analysis shows involvement in TNF, VEGF, IL-17, and NF-κB signaling pathways. P2RX7 contributes to inflammation and cytokine secretion (IL-1β, IL-18)[42]. IFN-β release is caused by pathogen-driven inflammation[43], PTGS2 and CTSK are involved in production of inflammatory mediators[44]. Since CTSK contributes to myocardial fibrosis, Voydeya targeting CTSK may reduce cardiac complications.

Zanubritinib targets inflammatory genes NTRK1, BDKRB1, and BDKRB2, showing highest binding with HSP90AA1 (-11.48 kcal/mol). Key pathways include inflammatory mediator regulation, IL-17 signaling, and MAPK signaling. The drug can reduce parasite persistence and neuronal dysfunction by targeting NTRK1 and modulating inflammatory response through BDKRB1/BDKRB2. HSP90AA1 aids protein folding in immune responses, playing crucial roles in cellular response to *T. cruzi* infection and immune cell recruitment[45]. From our computational analysis, the genomics targets and molecular pathways of these 5 drugs are found to be effective against *T. cruzi* infection. However, the pharmacokinetic property of the drugs must be validated with *in-vivo* experiments to further confirm the effectiveness of the drugs. Also Limited availability of *T. cruzi*-specific protein structures necessitated homology modeling.

Conclusion

In this study an integrated network pharmacology and molecular docking analysis was carried out to identify potential drug candidates among 20 FDA approved orphan drugs. From our initial screening, molecular docking of drugs with *Trypanosoma cruzi* glucokinase identified 13 drugs with good binding affinity. Further network pharmacological analysis revealed the possible molecular mechanism of 5 drugs namely Belumosudil, Quizartinib, Vomorolone, Voydeya and Zanubritinib in chagas disease pathogenesis. Almost all the drugs were found to modulate the inflammatory response of chagas disease by involving in pathways of inflammatory cytokines like NF-κB, IL-17 and TNF, which was again confirmed with correlating the results with series of case reports. Some targets like MMPs, MTOR, IL-6 are found to be involved in both cardiac and inflammatory phase of the disease. Final docking analysis with highly interacting genes of the network and drugs revealed their potential mechanism of action. From our results, we conclude that

Belumosudil and Voydeya can potentially target MMPs, Quizartinib can target CTSB, Vomorolone can target IL-6 and Zanubritinib can target HSP90 heat shock protein effectively to exhibit their molecular mechanism against *T. cruzi* infection. Case reports substantiate the critical role of MMPs and Interleukin genes in chagas disease progression, aligning with our network pharmacological findings.

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Conflict of interest

All authors declare no conflict of interest.

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