



Molecular Docking and Computational analysis of anti-parasitic drug molecules against Trypanosoma cruzi – Causative agent of Chagas Disease

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Abstract

Chagas disease, also known as Trypanosomiasis, is found mostly in Latin America by the infection of a parasite, Trypanosoma cruzi. It is considered to be vector born disease which is transmitted by an insect, Triatominae Bug. Other sources of transmission of disease are Blood transfusion, oral and Congenital transmission. According to WHO, it is the most ignored disease of tropical countries despite of the fact that more than 7 million people are found infected by it across the globe every year. Chagas Disease is listed in the group of twenty Neglected Tropical Diseases (NTDs). The study presented here is an attempt to explore potential inhibitors against Trypanosoma Cruzi by targeting the receptor protein, Cruzain which has a significant role in mechanism of infection and survival of this parasite inside the host cells. Cruzain, the primary cysteine protease of Trypanosoma cruzi, is critical for survival of the parasite in host cells, rendering it as an important target for development of inhibitors. Cruzain has been related with parasite metabolism and identified as both an important candidate for vaccine development and for trypanocidal drug design. Recent observations suggest that sulfonamides and hydrazide derivatives possess anti-parasitic activity and holds promise as anti trypanosomal chemotherapeutic agents suggesting their use as inhibitors to the protease. In this study, around five hundred sulfonamides and hydrazide derivatives and their similar conformers were screened using virtual screening and ultimately 50 conformers were chosen on the basis of the structural specificity to the enzyme towards its substrate and inhibitors. Using an exhaustive virtual-screening, docking and ADMET study in our work, we have identified 4-tert-butyl-N-[2-[4-(furan-2- carbonyl)piperazin-1-yl]-2-oxoethyl]benzene sulfonamide as the most potential efficient lead molecule against cruzain protein to treat chagas disease. Binding affinity of this molecule was -7.2 Kcal/mol which shows highly stable complex and it has exhibited drug likeness score of 0.78 and TPSA value of 86 with high potential for absorption in GI tract with no associated toxicity, mutagenicity, reproductive toxicity risk and has shown a great blood brain penetration coefficient.

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KeyWords: Insilico analysis, Chagas disease, Trypanosoma Cruzi, Cruzain Protein, Benzene sulfonamide, Drug designing, Virtual Screening, Molecular Docking

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Introduction

Chagas disease is a life threatening illness, transmitted through contact with faeces and urine of Triatomine bug (Kissing bug) found mainly in Latin America. Trypanosoma is a genus of

kinetoplastids which derive its name from greek-trypano(borer) and soma(body) because of their corkscrew-like motion.

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It is a species of parasitic Euglenoid which feeds on blood and lymph. It requires two intermediary host in its whole life cycle i.e Invertebrate host(Triatominae Bug) and Vertebrate host (Humans). Out of the three known species of Trypanosoma – Trypanosoma Brucei gambiense and Trypanosoma Brucei rhodesiense caused African Trypanosomiasis and Trypanosoma Cruzi causes Chagas disease. T. Cruzi is an intracellular parasite and for viability and proliferation relies on biosynthesis of endogenous sterols (JH Mckerrow et al, 2009).The two types of congenital Chagas disease are found , they are: Parasite seen within skeletal and cardiac fibres.Parasite is found within cells of the reticuloendothelial system (Achiléa Lisboa Bittencourt, 1976) . The discovery of treatment for Chagas disease has been reported as challenging by a number of research groups as there is scarcity of the information available about the factors responsible for this disease and also about the interaction between the parasite (Causing the disease) and the host (Jadel MüllerKratz, 2019). Trypanosoma Cruzi infects the person in two phases which includes acute and chronic phases. Acute Phase lasts for two months after infection. During this phase, the symptoms are either mild or absent in the patients. Initial symptoms observed are the skin lesions or purplish swelling of lids of one eye after the bug bites the person. In the Chronic Phase, the parasites are generally hidden in heart or digestive muscles. Hence, approximately 10% of people in chronic phase suffers with digestive or neurological alterations and 30% with cardiac disorders. After infecting the host, T. Cruzi affects a number of important systems such as digestive system and lymphatic system which causes immune responses by CD4+ and CD8+ which in turn has severe results such as blockage of cardiac system and prominent fibrosis(José Rodrigues Coura, 2007). The discovery of treatment for Chagas disease has been reported as challenging by a number of research groups as there is scarcity of the information available about the factors responsible for this disease and also about the interaction between the parasite (Causing the disease) and the host (Jadel MüllerKratz, 2019). In the olden times, Detection of Chagas disease was controversial as the traditional methods of diagnosis which includes conducting blood tests to detect the antigen have failed to find the traces of presence of this parasite in the blood stream of the infected individuals. However, this

issue has been addressed by PCR- based diagnostic techniques where it has been reported that the parasite is found hidden in the heart or digestive system (Julie Clayton,2010). Also an indirect Enzyme Based Immunosorbent assay (ELISA) detection found that there is no significant correlation between antibody titer (produced against disease) and cardiac or gastrointestinal tract disease (Saliva Elisa, 1999). The existence of T.Cruzi in Triatoma infestans also increases the defecation amount and frequency which increases the probability of contact between mammals and its infected feces (N. Pereyra et al, 2020). T.Cruzi infects by wounding the host cell ,which in turn initiates the signaling of surface receptors. By this the calcium ions in the host cell increases and parasites internalize in it .Its retention in cells is guaranteed by fusion of lysozyme with newly formed vacuoles of other cells. AS LAMP-1 and LAMP-2 helps in parasitophorous vacuoles formation (Albertti LA et al, 2010) so cells lacking LAMP-1 and LAMP- 2 are less prone to infection by T.Cruzi. It has been observed that the absence of LAMP-2 could decrease the chances of infection (McKerrow JH. et al, 2009) . A group of highly glycosylated protein, T.Cruzi mucin TcMUC which is rich in Proline, Serine and Threonine residues, is found to be responsible for invasion of host cells (Melo-Braga et al, 2022).As T. Cruzi relies on endogenous sterols for survival, there are a number of ergosterol inhibitors available but from experimental animal models and human patients it was found to be only successful in treating fungal diseases and not Chagas disease. Further, studies show that Chagas disease can be treated using drugs such as Nifurtimox (Nf) and Benznidazole (Bz) but these drugs have their own side effects and are only found to be suitable for patients below 40 years of age. Benznidazole is found to cause significant side effects which includes fever, muscular and articular pain, skin rashes. Like Benznidazole, Nifurtimox too causes side effects such as nausea, vomiting, weight loss and also certain tissue abnormalities in colon, mammary glands, ovaries and 15 testicles((JH Mckerrow et al, 2009). Hence, the side effects which are caused by the known treatments and drugs it is found to be extremely important to look for a better inhibitor which could be used as a drug. It has also been noted by WHO a complete treatment regime within 60 days is needed for better cure of this disease but both Nf and Bz are currently investigated to

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provide better results between 60-120 days (JH Mckerrow et al, 2009). The S2 pocket of Cruzain shows structural adaptation for both basic and functional groups and the inhibitors for S2 pocket of Cruzain protein places tyrosine and arginine in its S2 pocket (Gillmor SA et al, 1997). After the in vitro evaluation of sulfonamide, it was found that they show properties of new anti-T. Cruzi agent (Virgilio Bocanegra-Garcia et al, 2012). The synthesized sulfonamides when tested biologically showed that it acts on the host factors helping in viral infection and its propagation (Chieh-Chien Lee, 2010). Out of the sulfonamides, aromatic having halogeno tail or methoxyphenacetamido tails are found to inhibit TcCA but considered weak inhibitors as they have less penetration capacity while some with heterocyclic compounds are found good to be used as inhibitors (Peiwen Pan et al, 2013; Ozlen Guzel-Akdemir et al, 2013). For the discovery of a new drug which could cure Chagas disease the knowledge of Target product profile (TPP) is needed which could either be obtained by phenotypic-based or target-based approach. Genome editing approaches using CRISPR-Cas9 have been used to modulate genes of infectious protozoan parasites (Soares Medeiros LC et al, 2017). Recent innovations in artificial intelligence and virtual reality are changing the landscape of drug discovery and can prove to provide an effective roadmap in treatment of Chagas disease (Fleming N , 2018). Mapping and exploring biologically active chemical space can accelerate the pre clinical drug discovery (Sittampalam GS et al, 2019). Three key derivatives of benzimidazole have shown to demonstrate high efficacy in the treatment of Chagas disease (Torricio F et al, 2018).

2. Materials and Methods

2.1 Receptor Preparation

The three-dimensional crystal structure of Cruzain protein found in Trypanosoma Cruzi was retrieved from Research Collaboratory Structural Bioinformatics - Protein Data Bank (PDB id:1u9q) (Bank H.M. et al, 2000) and downloaded in .pdb format. Biovia Discovery studio software (BIOVIA, Dassault Systèmes, 2020) was applied to process and prepare the cruzain receptor protein for molecular docking in subsequent stages. During the processing of this receptor, the ligand, alpha ketoester, water molecules and all the hetero atoms were removed and polar hydrogen was added to it.

2.2 Virtual Screening for Retrieving Potential Ligands against Chagas Protein

Three Dimensional structure of Key ligands, Benzenesulfonamide and Iodo methyl sulfonamide were downloaded in SDF format from Pubchem database (Kim, S. et al, 2019) which is a widely used database for high throughput screening of millions of lead molecules. Using Benzenesulfonamide and Iodo methyl sulfonamide as the starting lead molecules, Pubchem database was explored to find out derivatives of these molecules and after screening a library of 500 potential compounds, 50 best lead molecules were selected based on the highest efficacy in terms of ADMET parameters as validated by Lipinski's rule (Lipinski, Christopher, 2004).

2.3 Molecular Docking Studies against Cruzain Protein

For the screening of anti-parasitic drugs all the fifty compounds (ligands) retrieved were analyzed in Auto dock Vina wizard (Morris, G. M et al, 2009; Dallakyan et al, 2015) and their binding affinity from nine different dimensions were recorded. The ligands with least binding affinities at Zero value of rmsd/ub and rmsd/lb were further assessed for ADMET parameters using SWISS ADME tool (Daina, A. et al, 2017) and OSIRIS property explorer (Sander, T, 2001).

2.4 Assessment of Pharmacokinetics Parameters for the Selected Molecules

For analyzing whether the selected ligands are suitable to be used as drug and are safe, various properties were analyzed such as the lipophilicity, solubility, toxicity, drug likeness, ability to cross Blood Brain barrier, digestion in GI tract, Bioavailability using SWISS ADME tool and The OSIRIS Property Explorer. SMILE notation of 50 ligands were used as an input in these two tools and Irritant, Tumorigenic, Mutagenic, Reproductive effect, drug likeliness, drug score, TPSA value, solubility were obtained.

3. Results

3.1 Virtual screening results

Pubchem database was explored to assess various derivatives of sulfonamides and hydrazide. Analysis of number of hydrogen bond donors, number of



hydrogen bond acceptors, molecular weight, partition coefficient between water and octanol to assess the potentiality of these hits as possible lead molecules was done and 50 derivatives (Table 1) were selected as lead molecules on the basis of extensive virtual screening.

Table 1 : Top 50 ligands screened after Virtual Screening applying Lipinski's Rule of Five

S.no	Pubchem id	Ligand name	Molecular weight	Log p	No. of Hydrogen Donors	No. of Hydrogen Acceptors
1	122044	4-tert-butyl-N-[6-(2-hydroxyethoxy)-5-(3-methoxyphenoxy)pyrimidin-4-yl]benzenesulfonamide	473.5	4	2	9
2	169682	4-(2-Aminoethyl)benzenesulfonamide	200.26	-0.2	2	4
3	5330790	4-({5-Amino-1-[(2,6-Difluorophenyl)carbonyl]-1h-1,2,4-Triazol-3-yl}amino)benzenesulfonamide	394.4	2.4	3	10
4	70018	4-(Trifluoromethyl)benzenesulfonamide	225.19	1.3	1	6
5	2950007	4-[3-(4-Methoxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]benzenesulfonamide	407.5	3.6	1	6
6	5312145	4-amino-N-[2,6-bis(methylamino)pyrimidin-4-yl]benzenesulfonamide	308.36	1.7	4	8
7	23642319	4-(5-(4-Chlorophenyl)-2-methyl-3-propionylpyrrol-1-yl)benzenesulfonamide	402.9	3.7	1	4
8	70701426	N-(1,3-benzothiazol-2-yl)-4-[(2-hydroxy-3-methoxyphenyl)methylamino]benzenesulfonamide	441.5	4.3	3	8
9	85485	Benzenesulfonamide, 4-[(5-cyano-1,2-dihydro-6-hydroxy-4-methyl-2-oxo-3-pyridinyl)azo]-	333.32	0.5	2	4
10	648990	Benzenesulfonamide, N-1H-benzimidazol-2-yl-	273.31	2.3	2	4
11	367783	4-[(3,4-Dioxo-3,4-dihydro-1-naphthalenyl)amino]benzenesulfonamide	328.3	1.3	2	6
12	9947165	4-(5-(4-chlorophenyl)-3-hydroxymethyl-1H-pyrazol-1-yl)benzenesulfonamide	363.8	1.9	2	5
13	101352	Benzenesulfonamide, N,N-bis(2-cyanoethyl)-	263.32	0.4	0	5
14	308411	4-((4-(Dimethylamino)benzylidene)amino)benzenesulfonamide	303.4	2.1	1	5
15	984193	4-tert-butyl-N-[2-[4-(furan-2-carbonyl)piperazin-1-yl]-2-oxoethyl]benzenesulfonamide	433.5	2.4	1	6
16	1370168	N-[4-oxo-5-(2-oxo-1H-indol-3-ylidene)-2-thiazolyl]benzenesulfonamide	385.4	2.4	2	7
17	1504737	N-benzyl-4-(4-isopropyl-2,3-dimethyl-5-oxo-2,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide	399.5	3.6	1	5
18	10835977	N-(2-Azepan-1-yl-ethyl)-4-iodo-N-methylbenzenesulfonamide	422.3	3	0	4

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19	59755640	4-Iodo-N-(2-methoxypyridin-4-ylmethyl)benzenesulfonamide	404.23	2.3	1	5
20	122353027	4-iodo-N-((6-methyl-2-(piperidin-1-yl)pyrimidin-4-yl)methyl)benzenesulfonamide	472.3	3	1	6
21	122353914	4-iodo-N-((6-methyl-2-morpholinopyrimidin-4-yl)methyl)benzenesulfonamide	474.3	1.8	1	7
22	690831	4-((4-Chlorobenzylidene)amino)benzenesulfonamide	294.76	2.4	1	4
23	9865808	4-(5-(4-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)benzenesulfonamide	401.8	3.7	1	7
24	7370	Benzenesulfonamide	157.19	0.3	1	3
25	1624667	N,N-dimethyl-3-[4-[(Z)-(4-oxo-2-sulfanylidene-1,3-thiazolidin-5-ylidene)methyl]-1-phenylpyrazol-3-yl]benzenesulfonamide	470.6	3.6	1	7
26	2985941	N-Ethyl-4-fluoro-N-(2-oxo-2-pyrrolidin-1-ylethyl)-benzenesulfonamide	314.38	1.6	0	5
27	25058452	4-Fluoro-3-((trifluoromethyl)sulfonyl)benzenesulfonamide	307.2	1.3	1	9
28	200818	Benzenesulfonamide, 4-amino-N-3-isoxazolyl-(9CI)	239.25	0.5	2	6
29	25039986	4-iodo-N-(4-methoxybenzyl)benzenesulfonamide	403.24	3	1	4
30	1327189	4-iodo-N-[(pyridin-3-yl)methyl]benzene-1-sulfonamide	374.2	2	1	4
31	290071	(4-Methoxy-2-methyl-3,7-dioxabicyclo[4.1.0]heptan-5-yl) acetate	202.2	0	0	5
32	1902656	5-iodo-2-methoxy-N-(pyridin-2-ylmethyl)benzenesulfonamide	404.23	2	1	5
33	120533	Benzenesulfonamide, 4-amino-N-(4-iodo-5-methyl-3-isoxazolyl)-	379.18	1.6	2	6
34	3811782	4-iodo-N-(1-methyl-4-piperidinyl)benzenesulfonamide	380.25	2.1	1	4
35	5741194	(2E)-2-(1H-benzimidazol-2-yl)-3-(1,3-dimethyl-6-nitro-2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)prop-2-enenitrile	374.4	2.1	1	5
36	91670078	4-iodo-3-methyl-N-(2,2,6,6-tetramethyl-4-piperidinyl)benzenesulfonamide	436.4	3.3	2	4
37	119087067	4-iodo-N-(1-methyl-1H-indazol-3-yl)benzenesulfonamide	413.24	3.1	1	4
38	121121714	4-iodo-N-((4-methyl-2-phenylthiazol-5-yl)methyl)benzenesulfonamide	470.4	4.2	1	5
39	121121812	4-iodo-N-((4-methyl-2-(thiophen-2-yl)thiazol-5-yl)methyl)benzenesulfonamide	476.4	3.9	1	6
40	121122444	4-iodo-N-((4-methyl-2-(pyridin-3-yl)thiazol-5-yl)methyl)benzenesulfonamide	471.3	3.1	1	6
41	122318436	4-iodo-N-((4-methyl-6-(piperidin-1-yl)pyrimidin-2-yl)methyl)benzenesulfonamide	472.3	3	1	6
42	122352613	4-iodo-N-((6-methyl-2-(pyrrolidin-1-yl)pyrimidin-4-yl)methyl)benzenesulfonamide	458.3	2.7	1	6



43	45481956	4-Iodo-N-((4-(methoxymethyl)-1H-1,2,3-triazol-5-yl)methyl)benzenesulfonamide	408.23	0.4	2	6
44	12231871 2	4-iodo-N-((4-(piperidin-1-yl)pyrimidin-2-yl)methyl)benzenesulfonamide	458.3	2.6	1	6
45	12235219 9	4-iodo-N-((2-(pyrrolidin-1-yl)pyrimidin-4-yl)methyl)benzenesulfonamide	444.3	2.3	1	6
46	57091867	N-(4-Iodo-benzyl)-benzenesulfonamide	373.21	3	1	3
47	10718443	N-(2-Azepan-1-yl-ethyl)-3-iodo-4-methoxy-N-methyl-benzenesulfonamide	452.4	2.9	0	5
48	94575	Benzenesulfonamide, p-(3,3-dimethyl-1-triazeno)-	228.27	1.1	1	6
49	22583789	4-iodo-N-(2-(4-methyl-2-(m-tolyl)thiazol-5-yl)ethyl)benzenesulfonamide	498.4	5	1	5
50	12231898 8	4-iodo-N-((4-morpholinopyrimidin-2-yl)methyl)benzenesulfonamide	460.3	1.4	1	7

3.2 Molecular Docking Results

Autodock Vina wizard in PyRx software was used for the receptor and ligand preparation whereby Heteroatoms and water molecules were deleted from the receptor, followed by addition of polar hydrogen and Kollman charges. Ligands were energy minimized and Steepest Descent algorithm was implemented for docking and energy minimization of 50 ligands selected. After assessing binding affinity and root mean square deviation in

upper and lower bound range of each possible 9 orientations generated for each of the 50 ligands, 10 ligands were selected (Table 2) on the basis of Binding affinity in the range -6.1 to -8.1. Further, different types of interactions (Hydrogen bonding, Vander waal interactions, Alkyl, Pi-Alkyl bonds) were assessed and analyzed for PubChem id:- 984193 and PubChem id :- 5330790 (Figure 1 and Figure 2).

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Table 2: 10 ligands selected based on Molecular Docking results of Autodock Vina

Ligands	Binding Affinity	rmsd/ub	rmsd/lb
1u9q1_70018_uff_E=478.03	-6.1	0	0
1u9q1_9865808_uff_E=946.43	-7.4	0	0
1u9q1_5330790_uff_E=1019.01	-7.7	0	0
1u9q1_23642319_uff_E=941.30	-7	0	0
1u9q1_5741194_uff_E=774.00	-7	0	0
1u9q1_122352613_uff_E=664.56	-7	0	0
1u9q1_122318712_uff_E=627.85	-7	0	0
1u9q1_122318436_uff_E=644.94	-7.1	0	0
1u9q1_1624667_uff_E=1170.57	-8.1	0	0
1u9q1_1370168_uff_E=1168.80	-7.6	0	0
1u9q1_984193_uff_E=877.56	-7.2	0	0
1u9q1_367783_uff_E=664.18	-7.8	0	0
1u9q1_648990_uff_E=775.95	-7	0	0



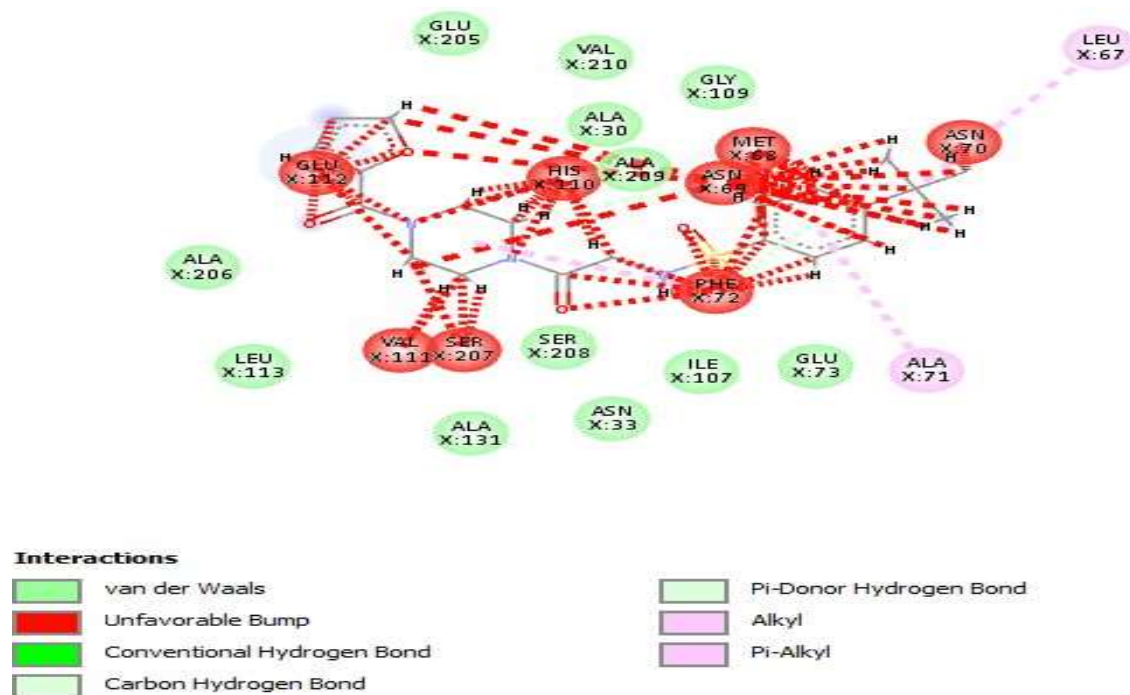


Fig 1 : Cruzain protein(PDB id :-1u9q) docked with 4-tert-butyl-N-[2-[4-(furan-2-carbonyl)piperazin-1-yl]-2-oxoethyl]benzenesulfonamide, PubChem id:- 984193(2D structure)

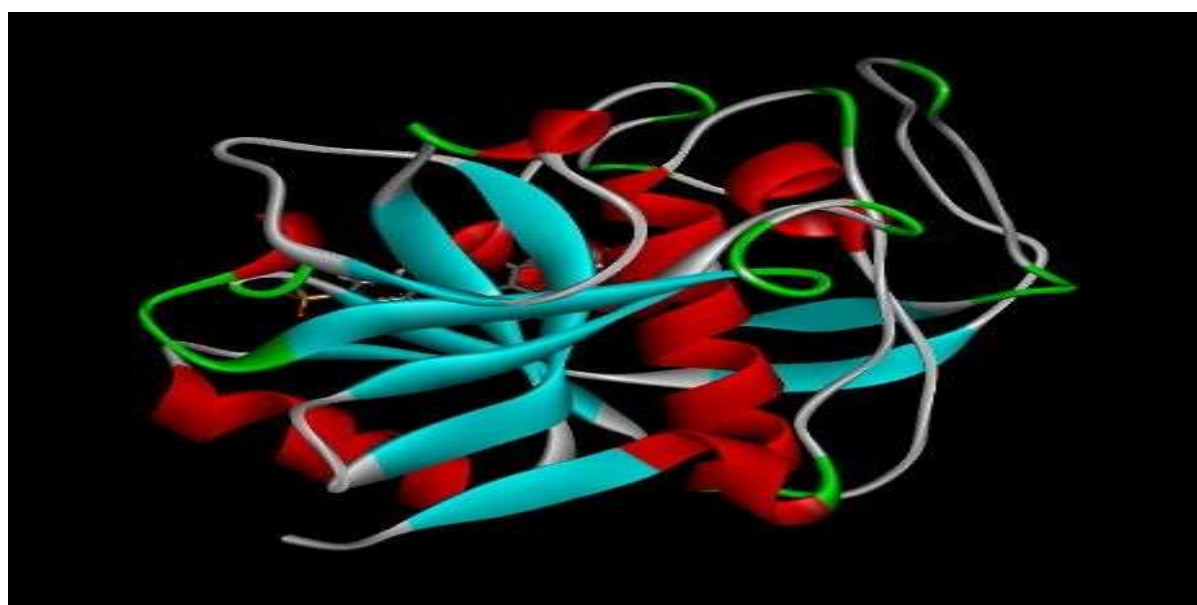


Fig 2: 1u9q docked with 4-tert-butyl-N-[2-[4-(furan-2-carbonyl)piperazin-1-yl]-2-oxoethyl]benzenesulfonamide

3.3 Toxicity assessment results

SWISS ADME tool was applied to assess TPSA score, Absorption in GI tract, possible side effects followed by using tool-OSIRIS Property Explorer to calculate mutagenicity, reproductive toxicity, tumorigenic potential, irritation potential of Top 10 ligands selected on the basis of molecular

docking results. Table 3 and Table 4 summarizes the results of SWISS ADME and OSIRIS Property Explorer. Finally, on the basis of these parameters, 4-tert-butyl-N-[2-[4-(furan-2-carbonyl)piperazin-1-yl]-2-oxoethyl]benzenesulfonamide was selected as the most potential anti parasitic drug molecule (Figure 3) against Cruzain protein in Trypanosoma Cruzi.



Table 3: Solubility, ClogP, TPSA and drug likeliness potential for promising ligand molecules identified against Cruzain protein

PubChem id	ClogP	Solubility	TPSA	Drug likeness	Drug Score
70018	1.27	-2.29	68.54	-6.2	0.47
9865808	2.85	-4.57	86.36	-6.41	0.34
5330790	1.91	-5.97	154.3	2.9	0.32
23642319	3.69	-7.66	90.54	5.79	0.42
5741194	1.34	-4.48	121.8	-9.46	0.37
122352613	2.39	-3.89	83.57	3.48	0.68
122318712	2.15	-3.3	83.57	2.04	0.69
122318436	2.55	-3.67	83.57	1.99	0.64
1624667	1.28	-4.15	150	2.64	0.65
1370168	1.48	-3.76	145.3	-2.39	0.43
984193	2.48	-2.44	108.3	5.09	0.78
367783	1.44	-3.61	114.7	-1.33	0.51
648990	1.93	-3.43	83.23	-5	0.44

Table 4: Toxicity assessment results for potential ligand molecules identified against Cruzain protein

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PubChem id	Mutagenic	Tumorigenic	Irritant	Reproductive Effective
70018	NO	NO	NO	NO
9865808	NO	NO	NO	NO
5330790	NO	NO	YES	NO
23642319	NO	NO	NO	NO
5741194	NO	NO	NO	NO
122352613	NO	NO	NO	NO
122318712	NO	NO	NO	NO
122318436	NO	NO	NO	NO
1624667	NO	NO	NO	NO
1370168	NO	NO	NO	NO
984193	NO	NO	NO	NO
367783	NO	NO	NO	NO
648990	NO	NO	NO	NO

The image shows a vertical sidebar from the SwissADME web tool. At the top, it says 'Molecule' with a red bar and a chemical structure icon. Below that, there are several sections of text: 'SMILES: O=C1CN2CCN(C2)CC1', 'Formula', 'Molecular weight', 'Num. heavy atom', 'Num. atom. bond', 'Fraction Csp3', 'Num. rotatable bond', 'Num. H-bond acceptor', 'Num. H-bond donor', 'Molar Refractivity', 'TPSA', and a list of partition coefficients: 'Log P_{ow} (ALIP)', 'Log P_{ow} (GLIP)', 'Log P_{ow} (DLIP)', 'Log P_{ow} (WLIP)', 'Log P_{ow} (MLIP)', 'Log P_{ow} (SLIP)', and 'Consensus Log P'.

Fig 3. SWISS ADME RESULTS FOR 4-tert-butyl-N-[2-[4-(furan-2- carbonyl)piperazin-1-yl]-2-



oxoethyl]benzenesulfonamide

4. Discussion And Conclusion

After analyzing all the selected ligands for Cruzain protein, the top ten ligands having lowest binding affinity were recorded. The analysis of ligands in SwissADME was done to assess water solubility, pharmacokinetics, accessibility, ability for crossing blood brain barrier, solubility in the GI tract, lipophilicity coefficient. Further, toxicity assessment was done different aspects i.e. Mutagenic, Tumorigenic, Irritant, Reproductive effect along with estimation of drug likeliness and drug score of all the selected ligands. It was found that 4-({5-Amino-1-[(2,6-Difluorophenyl)carbonyl]-1H-1,2,4-Triazol-3 Yl}amino) benzene sulfonamide, PubChem id :-5330790 shows irritant effect, hence it is not suitable for being used as a drug. Other ligands were found to be free of any toxicity effect, hence were proceeded for further analysis. As the compound with high value of clog p have poor absorption hence, the compound with lowest clog p value was found and it was 4-(Trifluoromethyl)benzenesulfonamide with PubChem id :-70018. As compound with TPSA (total polar surface area) value below 140 are considered to show good intestinal absorption and below 60 shows good blood-brain penetration, our analyses shows that all compound except N-[4-oxo-5-(2-oxo-1H-indol-3-ylidene)-2-thiazolyl]benzenesulfonamide. The highest solubility was found to be of ligand 4-(Trifluoromethyl)benzenesulfonamide (PubChem id :70018).

Best candidate based on the outcomes of docking using Autodock vina was 4-tert-butyl-N-[2-[4-(furan-2-carbonyl)piperazin-1-yl]-2-oxoethyl]benzenesulfonamide which showed binding affinity with free energy value of -7.2 kcal/mole and it exhibited TPSA score of 86.36 which reflects its high bioavailability and potential for optimum intestinal absorption and moderate blood brain barrier crossing capacity. Further, it exhibited no side effects and a suitable drug score of 0.78 and has been shown to cause non irritant along with no mutagenic or carcinogenic potential. Further, wet lab and experiment trials are recommended using animal cell lines to assess the MIC50 values along with actual mutagenic or genotoxic potential of this compound.

As of now, lot of emphasis has been laid on drug discovery using phenotypic traits for finding

potential drug molecules against Chagas disease (Swinney DC, Lee JA, 2020). In this context, appropriate combinatorial chemistry approaches for screening cascades have been applied intensively. A range of new promising inhibitors that block *T. cruzi* infection at very low concentrations are being deciphered and key targets for new inhibitors are being identified. Synergistic application and focus on Ergosterol pathway and phenotypic-based drug discovery can prove to be a gamechanger for treatment of Chagas disease in the coming times.

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Conflict of Interest

The authors declare no conflict of interest.

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