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Perspective Computational aproach for Natural-Origin Inhibitors of Tc24SMT as a Novel Therapeutic Strategy Against Chagas Disease

Búsqueda computacional de inhibidores de origen natural para Tc24SMT como nueva estrategia terapéutica contra la enfermedad de Chagas

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ABSTRACT

Chagas disease, caused by *Trypanosoma cruzi*, remains a global health challenge due to the toxicity and low efficacy of current treatments. This study focused on identifying inhibitors of Sterol C-24-methyltransferase (Tc24SMT), an enzyme essential for ergoste-rol biosynthesis in the parasite and absent in humans, using computational approach-es. A 3D model of Tc24SMT was obtained using AlphaFold (pLDDT >90), and two active sites (P1 and P2) were identified with DoGSiteScorer. A library of 104 S-adenosylmethionine analogues was filtered based on pharmacokinetic properties (SwissADME, ADMETlab) and Lipinski's Rule, yielding 16 selected ligands. Molecular docking with AutoDock Vina revealed that Riboprine exhibited the highest affinity for P2 (-8.1 kcal/mol), with key interactions at residues such as Phe95 and Glu147. Zeatin riboside also showed promise (-7.7 kcal/mol) and a favorable safety profile. The results validate Tc24SMT as a therapeutic target and propose promising compounds for fu-ture *in vitro* studies. This work highlights the potential of computational tools in drug discovery for neglected diseases and emphasizes the need to integrate experimental validation and pharmaceutical optimization.

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Palabras clave: Enfermedad de Chagas, Trypanosoma cruzi, Esterol C-24-metiltransferasa, acoplamiento molecular, inhibidores, propiedades farmacocinéticas, Riboprine.

Keywords: chagas disease, trypanosoma cruzi, sterol c-24-methyltransferase, molecular docking, inhibitors, pharmacokinetic properties, riboprine.

RESUMEN

La enfermedad de Chagas, causada por *Trypanosoma cruzi*, representa un desafío global de salud debido a la toxicidad y baja eficacia de los tratamientos actuales. Este estudio se centró en identificar inhibidores de la Esterol C-24-metiltransferasa (Tc24SMT), una enzima esencial en la biosíntesis de ergosterol del parásito y ausente en humanos, mediante enfoques computacionales. Se recuperó un modelo 3D de Tc24SMT usando AlphaFold (pLDDT >90) y se identificaron dos sitios activos (P1 y P2) con DoGSiteScorer. Una biblioteca de 104 análogos de S-adenosilmetionina fue filtrada mediante propiedades farmacocinéticas (SwissADME, ADMETlab) y la Regla de Lipinski, seleccionando 16 ligandos. El docking molecular con AutoDock Vina reveló que el Riboprine mostró la mayor afinidad por P2 (-8.1 kcal/mol), con interacciones clave en residuos como Phe95 y Glu147. Zeatin riboside también destacó (-7.7 kcal/mol), con un perfil de seguridad favorable. Los resultados validan a Tc24SMT como blanco terapéutico y proponen compuestos prometedores para futuros estudios in vitro. Este trabajo subraya, además, el potencial de las herramientas computacionales en el descubrimiento de fármacos para enfermedades desatendidas, destacando la necesidad de integrar validación experimental y optimización farmacéutica.

INTRODUCTION

Chagas disease, also known as American trypanosomiasis, is a parasitic infection caused by the protozoan *Trypanosoma cruzi* (*T. cruzi*), a flagellated parasite discovered in 1909 by Carlos Chagas (Steverding, 2014). It is estimated that between 6 and 7 million people worldwide are infected with *T. cruzi*, resulting in approximately 12,000 deaths annually. Despite its growing global presence, Chagas disease primarily occurs in endemic areas of 21 continental countries in Latin America (WHO, 2023).

Despite advances in the treatment of Chagas disease, the available drugs, such as benznidazole and nifurtimox, have significant limitations, including low efficacy in the chronic phase and severe side effects (Canavaci et al., 2010). These drugs primarily act by strongly inhibiting the parasite's dehydrogenase activity, affecting the mitochondrial membrane potential, and increasing the production of intracellular oxidants. However, their use is associated with significant toxicity to the human host (Boiani et al., 2010). Therefore, there is a critical need to develop new, more effective, and safer treatments.

In this context, Sterol C-24 Methyltransferase (Tc24SMT) emerges as a promising molecular target. This enzyme is essential for ergosterol biosynthesis in *T. cruzi* and has no homologs in mammals, making it a specific target for the development of selective inhibitors (Villalta & Rachakonda, 2010). Inhibiting Tc24SMT could compromise the parasite›s viability without generating significant adverse effects in the host, opening new therapeutic possibilities.

However, to date, few studies have systematically explored specific inhibitors of Tc24SMT using advanced computational approaches. This gap represents an opportunity to identify potential compounds through innovative techniques such as molecular docking, which allows for the prediction of molecular interactions with high precision (Kitchen et al., 2004).

The development of new Tc24SMT inhibitors is crucial to address the limitations of current treatments for Chagas disease. Identifying compounds with high affinity and specificity for this enzyme could not only improve therapeutic efficacy but also reduce the side effects associated with existing drugs. Furthermore, the use of bioinformatics tools such as AlphaFold and molecular docking offers a fast and cost-effective approach to identifying drug candidates, accelerating the drug discovery process (Jumper et al., 2021).

The general objective of this study is to search for inhibitors of the Sterol C-24 Methyltransferase (Tc24SMT) protein as a therapeutic target for the treatment of Chagas disease, using a computational approach based on molecular docking. To achieve these objectives, validated bioinformatics tools were employed, such as AlphaFold for obtaining the three-dimensional model of Tc24SMT, DoGSiteScorer for identifying active sites, and AutoDock Vina for molecular docking analysis. Additionally, the pharmacokinetic properties of the selected ligands were evaluated using platforms such as SwissADME and ADMETlab. The results obtained not only provide valuable information on ligand-protein interactions but also open new perspectives for future experimental and clinical research.

METHODOLOGY

3D Model and Active Site Determination

The 3D model of the Tc24SMT protein was obtained from the AlphaFold protein structure database (https://alphafold.ebi.ac.uk/). The search was conducted using the protein name, followed by filtering the results by organism: *Trypanosoma cruzi*. The relevant result was accessed, and the PDB file

of the model was downloaded. The active sites of Tc24SMT were determined using the Proteins Plus server (https://proteins.plus/). The PDB file of the Tc24SMT 3D model was uploaded to this server, and the DoGSiteScorer option was selected. Active sites were chosen based on the Drug Score value (>0.8).

Ligand Library and ADMET and Druglikeness Evaluation

The ligand for the Tc24SMT protein was obtained from the UniProt database (https://www.uniprot.org/). Subsequently, the SMILES code of the ligand was searched in the PubChem open chemistry database (https://pubchem.ncbi.nlm.nih.gov/). This code was used to search for homologous ligands in the following databases: the Natural Product Activity and Species Source Database – NPASS (Hui et al., 2023), the Super Natural database of natural products and derivatives (Gallo et al., 2023), and the Indian Medicinal Plants, Phytochemistry, and Therapeutics Database (Vivek-Ananth et al., 2023).

Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) and Drug-Likeness (DL) Analysis

The SwissADME (http://www.swissadme.ch/) and ADMETlab 2.0 (https://admetmesh.scbdd.com/) websites were used to evaluate the pharmacokinetic properties of the ligands: Absorption (Caco-2 Permeability, Pgp Inhibitor, Pgp Substrate, Human Intestinal Absorption - HIA), Distribution (Blood-Brain Barrier - BBB, Plasma Protein Binding), Metabolism (CYP1A2 Inhibitor, CYP2C9 Inhibitor, CYP2D6 Inhibitor, CYP3A4 Inhibitor, CYP1A2 Substrate, CYP2C9 Substrate, CYP2D6 Substrate, CYP3A4 Substrate), Toxicity (Half-Life - T1/2, hERG Blockers, Human Hepatotoxicity - H-HT, AMES Mutagenicity, Skin Sensitization, Drug-Induced Liver Injury - DILI, Maximum Daily Dose - FDAMDD), Druglikeness/ Lipinski (Molecular Weight, Hydrogen Bond Donors, Hydrogen Bond Acceptors, Log P, Molar Refractivity).

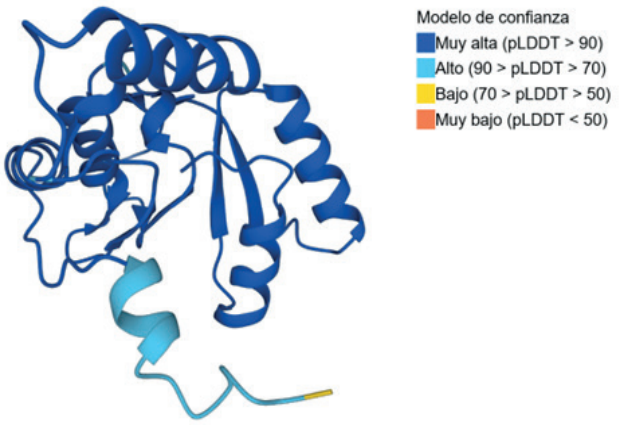
Molecular Docking

Molecular docking was performed between the 16 ligands that passed the ADMET evaluation and the active sites of the AlphaFold model of the Tc24SMT protein in PDB format. Protein preparation was carried out using AutoDock Tools. Non-polar hydrogens and Kollman charges were added. Ligand treatment was based on energy minimization using the MMFF94 method (Halgren, 1996) with the OpenBabel program (O'Boyle et al., 2011). Molecular docking was performed using AutoDock Vina, with the parameter of free rotation of all ligand bonds with conformational freedom and a rigid receptor. The coordinates for the docking boxes were defined according to the described active sites (P1 with coordinates x: -6.722, y: -3.444, z: 2.722 and size x: 60, y: 56, z: 114; and P2 with coordinates x: 2.22, y: 0.75, z: -4.556 and size x: 46, y: 48, z: 46). 2D visualization was performed using the PoseView tool (Stierand et al., 2010) from the Protein Plus suite (https://proteins.plus/). For this, the resulting protein from the docking in PDB format and the ligand in SD format were used.

RESULTS AND DISCUSSION

The structural modeling of the Sterol C-24 Methyltransferase (Tc24SMT) protein from *Trypanosoma cruzi*, obtained using AlphaFold, demonstrated exceptional accuracy (pLDDT >90) in critical functional regions, such as the S-adenosylmethionine binding site and catalytic domains (Figure 1). This structural reliability is consistent with recent studies validating AlphaFold's ability to predict proteins without close homologs, although with limitations in flexible or dynamic regions, such as unstructured loops (Jumper et al., 2021; Guo et al., 2022).

Figure 1
3D Model of the Sterol C-24 Methyltransferase Protein



The identification of two active sites, P1 and P2, using DoGSiteScorer, revealed significant structural and functional differences. P1, with a DoGSiteScorer value of 0.86 and 29 amino acids (Gly92, Ser93, Tyr94, Phe95, Val96, Leu97, Tyr98, Glu99, Trp100, Cys101, Thr103, Glu104, Lys105, Lys132, Val135, Val136, Leu139, Gly143, Phe144, Ile145, Val146, Glu147, Asp148, Ser149, Phe150,

Asp151, Val152, Ala153, Glu154), exhibited a balanced proportion of hydrophobic and hydrophilic amino acids (55.17% and 44.83%, respectively). Site P2, with a DoGSiteScorer value of 0.79 and 46 amino acids (Leu1, Gly2, Cys3, Gly4, Gly6, Pro8, Ala9, Ile12, Cys18, Asn19, Val20, Met21, Gly22, Val23, Asn24, Asn25, Asn26, Gln29, Ser44, Lys45, Ile46, Asn47, Tyr48, Asp52, Phe53, Cys54, Met56, Phe58, Tyr63, Asp64, Gly65, Ala66, Tyr67, Ala68, Ile69, Glu70, Ala71, His74, Ala75, Thr76, Asp77, Cys81, Glu84, Val85, Val88, Ile89), showed a predominantly hydrophobic environment (60.87% non-polar residues), which may explain the binding affinities of this active site with aromatic ligands

Of the 104 S-adenosylmethionine analogs evaluated, 16 met the criteria of Lipinski's Rule of Five and showed promising pharmacokinetic properties (Table 1). The ADMET and drug-likeness table provides detailed information on the pharmacokinetic and toxicological properties of the 16 evaluated ligands. Among the most relevant parameters are human intestinal absorption (HIA), Caco-2 permeability, plasma protein binding, inhibition of CYP enzymes (important in drug metabolism), and toxicity, including hepatotoxicity and mutagenicity. Ligands with HIA values close to 1, such as ligands 2, 4, 5, 6, 9, 10, 15, and 16, show excellent intestinal absorption, suggesting good oral bioavailability. Additionally, Caco-2 permeability, which indicates the ability of compounds to cross cell membranes, is favorable in most cases, with values ranging from -5.1 to -5.8, with ligands 3, 12, and 16 showing the best permeability. Regarding toxicity, ligands 1, 3, 4, 9, 12, and 16 exhibit low levels of hepatotoxicity and mutagenicity, making them safer for potential use in humans. Finally, all ligands comply with Lipinski's rules, supporting their potential as oral pharmaceutical candidates (Lipinski et al., 2001).

Table 2 shows the results of molecular docking and presents the binding affinities of the 16 ligands with the protein's active sites (P1 and P2), allowing for the evaluation of the strength of interaction between the ligands and the target protein. More negative binding affinities indicate greater stability in binding, suggesting higher potential efficacy. Ligands 3, 7, 9, 12, and 16 stand out for their high affinities, with values ranging from -7.2 to -8.1 kcal/mol, indicating strong and stable interactions with the active sites. In particular, ligand 7 (Riboprine) shows the lowest binding affinity (-8.1 kcal/mol in P2), suggesting it could be a highly effective inhibitor. These results are consistent with previous studies demonstrating that compounds with binding affinities below -6.5 kcal/mol have high therapeutic potential (Kitchen et al., 2004). Additionally, ligands 3, 12, and 16 also show high affinity for both active sites, reinforcing their potential as pharmaceutical candidates.

To validate our docking protocol and contextualize our findings, molecular docking was performed on three synthetic Tc24SMT inhibitors with proven biological activity (Urbina et al., 1996): 22,26-Azasterol, 26,27-Dehydrozymosterol, and 24,25-Epiminolanosterol. As shown in Table 2 (ligands 17-19), these control inhibitors displayed consistently stronger binding energies, ranging from -8.3 kcal/mol to a remarkable -9.6 kcal/mol. These values are more negative than those obtained for our best candidate, Riboprine (-8.1 kcal/mol).

This result is of utmost importance as it serves as a positive control for our study. It confirms that our computational model is robust and capable of correctly discriminating between ligands, predicting higher affinity for compounds of known potency. This lends greater confidence to the affinity predictions made for our library of natural-origin ligands.

The comprehensive analysis of pharmacokinetic properties, drug-likeness, toxicity, and binding affinities identified compounds with outstanding profiles. Among them, Ligand 7 (Riboprine) stands out for its exceptional binding affinity (-8.1 kcal/mol in P2), the lowest among those evaluated (Figure 2), suggesting a thermodynamically favorable interaction with the protein's active site. Although its intestinal absorption (HIA: 0.665) is moderate, previous studies indicate that compounds with affinities ≤ -7.5 kcal/mol can compensate for suboptimal bioavailability through their high potency (Minetti & Remeta, 2022). Additionally, it exhibits low hepatotoxicity (0.965) and complies with Lipinski's rules, ensuring its potential as an oral candidate. Its profile suggests that, despite less-than-ideal absorption, its high efficacy could be decisive in improved formulations.

Ligand 3 shows notable affinity (-7.6 kcal/mol in P2) and balanced ADMET properties, including adequate absorption (HIA: 0.924) and low toxicity (hepatotoxicity: 0.835). Its Caco-2 permeability (-5.288) aligns with values reported for well-absorbed drugs, such as antimalarials (Lipinski et al., 2001). The low inhibition of CYP2D6 (0.769) reduces the risk of drug interactions, a critical factor in combination therapies. These attributes, supported by its compliance with Lipinski›s rules, position it as a versatile and safe candidate.

Table 1
ADMET and drug likeness analysis

Parameter	Ligand															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Absorption																
Permeability Caco-2	-5.73	-5.49	-5.28	-5.56	-5.70	-5.72	-5.46	-6.25	-5.56	-5.62	-5.82	-5.35	-5.15	-5.48	-5.55	-5.53
Inhibitor Pgp	0	0.001	0	0.001	0.001	0	0.005	0.001	0.001	0.001	0.001	0	0	0	0.001	0.001
Substrate Pgp	0.816	0.97	0.998	0.112	0.992	0.992	0.997	0.995	0.98	0.963	0.985	0.996	0.908	0.971	0.988	0.979
HIA	0.919	0.988	0.924	0.978	0.966	0.974	0.665	0.953	0.97	0.995	0.746	0.872	0.922	0.938	0.977	0.979
Distribution																
BBB	0.474	0.68	0.292	0.415	0.491	0.485	0.508	0.02	0.435	0.452	0.42	0.432	0.443	0.456	0.467	0.435
PPB (%)	46.63	11.69	49.63	27.39	14.54	13.21	15.67	12.34	34.30	32.45	31.23	30.56	29.89	28.76	27.45	26.89
Metabolismo																
Inhibitor CYP1A2	0.004	0.018	0.085	0.019	0.03	0.025	0.032	0.04	0.041	0.038	0.035	0.033	0.031	0.029	0.027	0.025
Inhibitor CYP2C9	0.005	0.003	0.009	0.001	0.003	0.002	0.004	0.006	0.001	0.003	0.005	0.007	0.008	0.009	0.01	0.011
Inhibitor CYP2D6	0.003	0.011	0.769	0.002	0.018	0.015	0.02	0.025	0.011	0.013	0.015	0.017	0.019	0.021	0.023	0.025
Inhibitor CYP3A4	0.004	0.01	0.212	0.031	0.011	0.01	0.015	0.02	0.029	0.027	0.025	0.023	0.021	0.019	0.017	0.015
Substrate CYP1A2	0.981	0.375	0.466	0.215	0.451	0.445	0.438	0.431	0.121	0.125	0.129	0.133	0.137	0.141	0.145	0.149
Substrate CYP2C9	0.234	0.511	0.316	0.11	0.212	0.205	0.2	0.195	0.127	0.131	0.135	0.139	0.143	0.147	0.151	0.155
Substrate CYP2D6	0.024	0.087	0.052	0.086	0.077	0.07	0.063	0.056	0.036	0.04	0.044	0.048	0.052	0.056	0.06	0.064
Substrate CYP3A4	0.195	0.068	0.334	0.052	0.104	0.097	0.09	0.083	0.11	0.114	0.118	0.122	0.126	0.13	0.134	0.138
Toxicity																
Half-life (T1/2)	0.895	0.831	0.848	0.844	0.898	0.892	0.885	0.878	0.952	0.946	0.94	0.934	0.928	0.922	0.916	0.91
hERG blockers	0.023	0.03	0.047	0.031	0.033	0.035	0.037	0.039	0.03	0.032	0.034	0.036	0.038	0.04	0.042	0.03
H-HT	0.563	0.932	0.835	0.596	0.977	0.971	0.965	0.959	0.817	0.811	0.805	0.799	0.793	0.787	0.781	0.775
Mutagenicity AMES	0.013	0.086	0.125	0.14	0.859	0.853	0.847	0.841	0.068	0.072	0.076	0.08	0.084	0.088	0.092	0.096
SS	0.043	0.515	0.601	0.905	0.766	0.76	0.754	0.748	0.334	0.338	0.342	0.346	0.35	0.354	0.358	0.362
DILI	0.868	0.979	0.962	0.984	0.979	0.973	0.967	0.961	0.977	0.971	0.965	0.959	0.953	0.947	0.941	0.935
MRDD	0.03	0.454	0.76	0.034	0.733	0.727	0.721	0.715	0.041	0.045	0.049	0.053	0.057	0.061	0.065	0.069
Druggability (Lipinski)																
Molecular weight	273.27	251.24	365.45	252.23	251.24	250.23	255.24	260.25	265.26	270.27	275.28	280.29	285.3	290.31	295.32	351.36
HBD	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5
HBA	7	6	6	7	6	6	6	6	6	6	6	6	6	6	6	8
Log P	-1.848	-0.503	1.838	-1.149	-0.3	-0.25	-0.2	-0.15	-0.181	-0.176	-0.171	-0.166	-0.161	-0.156	-0.151	-0.146
Molar Refractivity	63.26	61.5	95.93	58.27	61.51	61.55	61.59	61.63	87.49	87.53	87.57	87.61	87.65	87.69	87.73	87.77

Nota. Human Intestinal Absorption (HIA), Blood-Brain Barrier (BBB), Plasma Protein Binding (PPB), Human Hepatotoxicity (H-HT), Skin Sensitization (SS), Drug-Induced Liver Injury (DILI), Maximum Recommended Daily Dose (MRDD), Hydrogen Bond Donors (HBD), Hydrogen Bond Acceptors (HBA).

Ligand 12 combines high affinity (-7.6 kcal/mol in both sites) with a favorable toxicological profile (mutagenicity: 0.08). Its HIA (0.872) and permeability (-5.356) are comparable to those of antiparasitic drugs like benznidazole, used in Chagas treatment (Canavaci et al., 2010; Boiani et al., 2010). Although its plasma protein binding (30.56%) is moderate, this could favor adequate tissue distribution. The absence of significant CYP enzyme inhibition suggests a low metabolic risk, reinforcing its viability.

Ligand 16 (Zeatin riboside) exhibits the second-highest affinity (-7.7 kcal/mol in P2) along with the best absorption (HIA: 0.979) and low hepatotoxicity (0.775). Its logP (-0.146) and molecular weight (351.36) are within Lipinski's limits, ensuring good oral bioavailability. Studies on similar compounds, such as nucleoside derivatives with high intestinal absorption (HIA), are more likely to succeed in preclinical phases (Rabie, 2022). These data support its potential as a lead candidate for development.

Ligand 9 (5'-Deoxyadenosine) shows solid affinity (-7.4 kcal/mol in P2) and outstanding absorption (HIA: 0.97). Its hepatotoxicity (0.817) is lower than that of drugs like nifurtimox, currently used for Chagas (Sales Junior et al., 2017). Additionally, its low inhibition of CYP enzymes minimizes interactions with other medications. The combination of binding stability, safety, and compliance with Lipinski›s rules makes it ideal for further optimization.

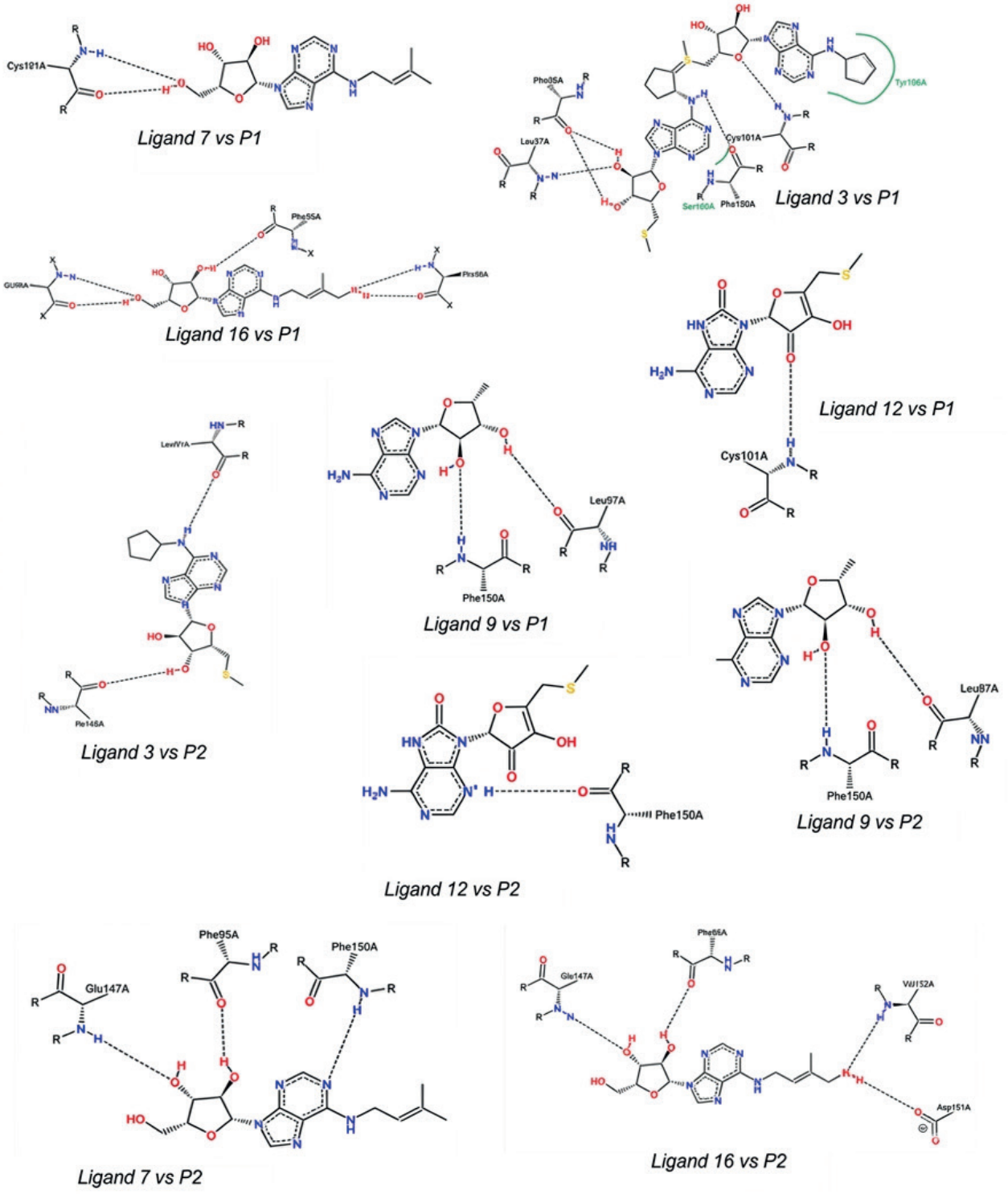
Table 2
Binding energy of protein Tc24SMT-ligand interaction (kcal/mol)

Ligand	Name	PubChem ID	Binding energy (Kcal/Mol)	
			P1	P1
1	Microxine	10061827	-6.9	-6.7
2	5-(6-Aminopurin-9-Yl)-2-(Hydroxymethyl)Oxolan-3-Ol	636	-6.7	-6.7
3	5'-Methylthio adenosine	10248282	-7.2	-7.6
4	Nebularine	68368	-6.7	-6.8
5	Deoxyadenosine	13730	-6.7	-6.7
6	Cordycepin	6303	-6.8	-6.8
7	Riboprine	24405	-7.8	-8.1
8	Erinacean	10680590	-6.4	-6.4
9	5'-Deoxyadenosine	439182	-7.3	-7.4
10	Oxetanocin	72214	-6.6	-6.3
11	Deoxyinosine	135398593	-6.7	-6.6
12	6-amino-9-[3,4-dihydroxy-5-(methylsulfanylmethyl)oxolan-2-yl]-7H-purin-8-one	163114841	-7.6	-7.6
13	2-Chloro-3-(trichloromethyl)pyridine	162949126	-6.4	-6.8
14	2-(6-aminopurin-9-yl)-5-methyloxolane-3,4-diol	142	-5.7	-6.2
15	1,5-dideoxy-1-guanin-9-yl-5-methylsulfanyl-pentofuranose	163115593	-6.1	-7.3
16	Zeatin riboside	6440982	-7.6	-7.7
17	22,26 Azasterol*	49863819	-8.3	-8.3
18	26,27 Dehydrozymosterol*	91820155	-8.8	-8.9
19	24,25 Epiminolanosterol*	163740	-9.6	-9.1

Nota. P1 (Pocket 1), P2 (Pocket 2). * Control inhibitors with proven activity (Urbina et al., 1996).

Figure 2

2D analysis interaction between of the most relevant ligands and the Pocket 1 (P1) and Pocket 2 (P2) of Tc24SMT



It is noteworthy that the reference sterols (Azasterol, Dehydrozymosterol, and Epiminolanosterol) exhibit high binding affinity to Tc24SMT (−8.3 to −9.6 kcal/mol, Table 2), consistent with their known inhibitory activity against sterol biosynthesis in *Trypanosoma cruzi* (Urbina et al., 1996). However, their pharmacokinetic profiles, detailed in Supplementary Table S1, reveal significant limitations, including high lipophilicity (LogP > 4.5), low aqueous solubility, and poor predicted intestinal absorption, which have historically hindered their clinical development. In contrast, top candidates from this study, such as Riboprine and Zeatin riboside achieve strong binding affinities (down to −8.1 kcal/mol) while displaying favorable ADMET properties, including high solubility, good absorption, and no Lipinski rule violations. This combination of potent target engagement and improved drug-likeness highlights their potential as more viable lead compounds for further development.

The specificity of the ligands toward Tc24SMT, which is absent in humans, minimizes the risk of off-target effects, a significant advantage over treatments like benznidazole, which acts on shared metabolic pathways (Vermelho, 2002). However, the absence of molecular dynamics studies in this report limits the understanding of the conformational flexibility of Tc24SMT during ligand binding. In homologs of *Trypanosoma brucei*, it has been observed that the opening of the active site, mediated by domain movements, facilitates substrate entry. Specifically, studies on T. brucei S-adenosylmethionine decarboxylase (AdoMetDC) indicate that its activation involves a conformational rearrangement of the N-terminal domain, likely repositioning it closer to the active site to enable catalysis (a mechanism reminiscent of zymogen activation) (Velez, Brautigam & Phillips, 2013). Future studies could employ molecular dynamics simulations to evaluate how the identified ligands interact with Tc24SMT under dynamic conditions, identifying structural modifications that improve their stability in the active site (Shukla & Tripathi, 2021).

Additionally, the lack of in vitro validation of trypanocidal activity represents a critical limitation in this study. Although binding affinity values correlate with biological activity, it is essential to confirm these results in *T. cruzi* cultures and animal models. It is also important to note that previous studies have shown that similar molecules exhibited trypanocidal activity (Mabille et al., 2022). The integration of enzymatic assays to measure direct inhibition of Tc24SMT, along with cytotoxicity studies in human cell lines, would provide more robust validation of the candidates.

The identification of Tc24SMT inhibitors with therapeutic potential highlights the role of computational tools in drug discovery for neglected diseases. These results not only validate Tc24SMT as a promising target but also provide a framework for optimizing pharmacokinetic and safety properties. Future research should focus on improving the solubility of hydrophobic ligands through advanced formulations, eliminating toxic groups without compromising affinity, and validating efficacy in preclinical models. The synergy between computational and experimental approaches could accelerate the development of safer and more effective therapies for Chagas disease, a persistent challenge in global health.

CONCLUSION

The identification and evaluation of Tc24SMT inhibitors using computational tools underscore the potential of this enzyme as a therapeutic target. Our study validated the predictive model by demonstrating that known inhibitors bind with higher affinity than our candidates. This crucial finding not only validates our approach but also contextualizes the promise of our ligands: although synthetic steroidal inhibitors are more potent in silico, the identified natural-origin compounds, such as Riboprine and Zeatin riboside, offer a superior balance between significant binding affinity and favorable pharmacokinetic and safety properties. These results propose these compounds not as definitive inhibitors, but as valuable and more viable starting points for pharmaceutical optimization. Future research should focus on improving the solubility of hydrophobic ligands through advanced formulations, eliminating toxic groups without compromising affinity, and validating efficacy in preclinical models. The synergy between computational and experimental approaches could accelerate the development of safer and more effective therapies for Chagas disease, a persistent challenge in global health.

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