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Virtual screening of natural products as potential inhibitors of triosephosphate isomerase of *Rhipicephalus microplus*

Triagem virtual de produtos naturais como potenciais inibidores da triosefosfato isomerase de *Rhipicephalus microplus*

Evaluación virtual de productos naturales como posibles inhibidores de la triosafosfato isomerasa de *Rhipicephalus microplus*

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ABSTRACT

Objective: In this study, molecular docking of 332 natural products selected from the ZINC15 database was performed on the three-dimensional structure of *R. microplus* TIM using Molegro Virtual Docker. **Methods:** The absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the best compounds were predicted. After molecular docking, the 20 compounds with the lowest MolDock Score, indicative of the highest predicted affinity to *R. microplus* TIM, were evaluated. **Results:** Fifty percent are categorized as alkaloids and aminoglycosides, 20% as dipeptides and terpenoids. Eighty percent comprise anticancer and antimicrobial compounds. Paclitaxel, dirithromycin, toposar, natamycin, and cabazitaxel exhibited the highest affinities for *R. microplus* TIM, with MolDock scores of -171.258, -168.586, -149.368, -148.880, and -148.810, respectively. **Conclusion:** Expanding research into TIM inhibition and modifying the studied compounds could thus lead to the discovery of new acaricides. This study's findings enhance our understanding of TIM inhibition in ticks, confirming its druggability as a target for natural compounds and aiding in the development of strategies for improved tick control.

Keywords: Ticks, Druggability, TIM inhibition, Molecular docking.

RESUMO

Objetivo: Neste estudo, o docking molecular de 332 produtos naturais selecionados do banco de dados ZINC15 foi realizado na estrutura tridimensional de *R. microplus* TIM usando o Molegro Virtual Docker. **Métodos:** As propriedades de absorção, distribuição, metabolismo, excreção e toxicidade (ADMET) dos melhores compostos foram previstas. Após o docking molecular, os 20 compostos com o menor MolDock Score, indicativo da maior afinidade prevista para *R. microplus* TIM, foram avaliados. **Resultados:** Cinquenta por cento são categorizados como alcaloides e aminoglicosídeos, 20% como dipeptídeos e terpenoides. Oitenta por cento compreendem compostos anticâncer e antimicrobianos. Paclitaxel, diritromicina, toposar, natamicina e cabazitaxel exibiram as maiores afinidades para *R. microplus* TIM, com pontuações MolDock de -171,258, -168,586, -149,368, -148,880 e -148,810, respectivamente. **Conclusão:** Expandir a pesquisa sobre a inibição de TIM e modificar os compostos estudados pode, portanto, levar à descoberta de novos acaricidas. As descobertas deste estudo aumentam nossa compreensão da inibição de TIM em carrapatos, confirmando sua drogabilidade como um alvo para compostos naturais e auxiliando no desenvolvimento de estratégias para melhor controle de carrapatos.

Palavras-chave: Carrapatos, Drogabilidade, Inibição de TIM, Docagem molecular.

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RESUMEN

Objetivo: En este estudio, se realizó el acoplamiento molecular de 332 productos naturales seleccionados de la base de datos ZINC15 sobre la estructura tridimensional de *R. microplus* TIM utilizando Molegro Virtual Docker. **Métodos:** Fueron evaluadas las propiedades de absorción, distribución, metabolismo, excreción y toxicidad (ADMET) de los mejores compuestos. Después del acoplamiento molecular, se evaluaron los 20 compuestos con el puntaje MolDock más bajo, indicativo de la afinidad más alta predecida por *R. microplus* TIM. **Resultados:** El cincuenta por ciento se clasifica como alcaloides y aminoglucósidos, el 20% como dipéptidos y terpenos. El ochenta por ciento comprende compuestos anticancerígenos y antimicrobianos. Paclitaxel, diritromicina, toposar, natamicina y cabazitaxel mostraron las mayores afinidades por la TIM de R. microplus, con puntuaciones de MolDock de -171,258, -168,586, -149,368, -148,880 y -148,810, respectivamente. **Conclusión:** La ampliación de la investigación sobre la inhibición de la TIM y la modificación de los compuestos estudiados podría conducir al descubrimiento de nuevos acaricidas. Los hallazgos de este estudio mejoran nuestra comprensión de la inhibición de la TIM en garrapatas, lo que confirma su capacidad de ser un objetivo para los compuestos naturales y ayuda al desarrollo de estrategias para un mejor control de las garrapatas.

Palabras clave: Garrapatas, Farmacología, Inhibición de TIM, Acoplamiento molecular.

INTRODUCTION

The tick *Rhipicephalus microplus* is the most significant ectoparasite in tropical and subtropical regions, causing annual economic losses estimated at US$ 3.2 billion (GRISI L, et al., 2014). Its bite induces dermal damage, leading to local inflammation that negatively impacts the livestock leather industry. Moreover, it adversely affects animal weight gain, thereby reducing meat production (JONSSON N, 2006; Nicaretta JE, et al., 2023). Additionally, *R. microplus* serves as the primary vector for various cattle-borne pathogens, including *Babesia* spp. and *Anaplasma spp.* (MIRABALLES C, et al., 2019; MARQUES R, et al., 2020).

Predominantly, synthetic acaricides are used to control these ectoparasites. However, continuous and indiscriminate use of acaricides has led to the emergence of chemically resistant tick populations (KLAFKE G, et al., 2017; AGWUNOBI DO, et al., 2021; WALDMAN J, et al., 2023). Therefore, the search for novel compounds with different mechanisms of action to exert effective acaricidal activity against *R. microplus* is crucial. Numerous chemical compounds have been explored for their potential in parasite control strategies, particularly those targeting specific parasite enzymes (BEZERRA WAS, et al., 2022; SAPORITI T, et al., 2022; MALAK N, et al., 2023).

Given that triosephosphate isomerase (TIM) is an enzyme involved in glycolysis and gluconeogenesis, processes that play a critical role in carbon and energy metabolism (KUMAR K, et al., 2012; LIANG N, et al., 2022), its potential as target for drug development against various parasites has been studied (BRAZ V, et al., 2019; JUÁREZ-SALDIVAR A, et al., 2021; GONZÁLEZ-MORALES LD, et al., 2023), including for TIM of *R. microplus* (SARAMAGO L, et al., 2018; MALAK N, et al., 2023). Despite the highly structural similarity of this enzyme between species, it has been possible to obtain selective inhibitors if they target the enzyme's dimer interface, as this region is poorly conserved (TÉLLEZ-VALENCIA A, et al., 2004).

Plant-derived products are attractive because of their generally low toxicity, limited environmental persistence, and complex chemical structure, which may hinder development of resistance resulting from modification of the compound (SELLES SMA, et al., 2021). Identifying and developing pharmaceuticals targeting essential tick enzymes, such as TIM, represent an innovative and promising approach to drug development.

In this context, natural products emerge as promising candidates for controlling *R. microplus* by targeting its TIM. Advancements in computational techniques have enabled the introduction of new virtual screening methods, providing a faster and cheaper alternative to in vitro screening of libraries of drug-like compounds (JUÁREZ-SALDIVAR A, et al., 2021).

These methods rely on the in-silico analysis and modeling of molecular interactions between potential ligands and target molecules (WADOOD A, et al., 2013; KIAMETIS et al., 2017), significantly contributing to

the identification of novel drug candidates (CHOUBEY SK, JEYARAMAN J, 2016; SARAMAGO L, et al., 2018; GANESAN M, et al., 2020). Given the scientific and economic significance of advancing novel acaricidal products against ticks, coupled with the pivotal role of TIM as a target enzyme essential to tick physiology, this study utilized in-silico techniques to evaluate potential natural products against *R. microplus* TIM.

METHODS

Structure of natural products and ADMET features

The structures of 332 compounds were obtained, in mol2 format, from the ZINC15 database using the filters: natural products, and for sale and world (approved drugs in major jurisdictions, including the FDA, i.e. DrugBank approved). The theoretical ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of compounds were analyzed using PreADMET software (KWANG LS, 2005).

Molecular docking of natural products onto triosephosphate isomerase (TIM) of *Rhipicephalus microplus*

To analyze the potential inhibitory activity of selected compounds to the *R. microplus* enzyme, molecular docking was carried out in Loop 3 of the dimer interface of the tick TIM, using Molegro Virtual Docker 6.0 (MVD) software. The structure of the TIM, at 2.40 Å resolution (PDB ID: 3TH6 – chain A), was obtained from the Protein Data Bank (www.rcsb.org).

The structures of natural products and TIM were imported into the MVD workspace in 'mol2' format. The enzyme structure was prepared (always assigning bonds, bond orders and hybridization, charges, and tripos atom types; always creating explicit hydrogens and always detecting flexible torsions in ligands) using the utilities provided in MVD. Molecular docking was carried out inside a virtual docking sphere of 15 Å radius and the following center coordinates X: 9.52; Y: 5.94; Z: -23.62 Å, using MolDock Score [GRID] as the score function and the MolDock SE as the search algorithm. Ten independent runs were conducted, and the results were expressed in the MolDock score. The more negative the number, the better the binding.

The 20 compounds exhibiting the lowest MolDock Scores were identified, and their scaffold, chemical classes, and biological classes were determined through analysis using the ZINC15 database. Subsequently, the five molecules with the lowest MolDock scores were selected for further examination. The optimal pose of each molecule with TIM was visualized and assessed utilizing the PyMOL Molecular Graphics System v1.3 (http://www.pymol.org). Additionally, the residues of TIM interacting with natural products were analyzed using the Discovery Studio Visualizer program v21.1.0 (https://www.3ds.com/products/biovia/discovery-studio).

RESULTS

Molecular docking

After molecular docking of structures of 322 natural products, selected from the ZINC15 database, onto the available, crystallographically-determined three-dimensional structure of *R. microplus* TIM, as described in the Methodology section, the 20 compounds with the lowest MolDock scores were retained (listed in Table 1). Alkaloids, aminoglycosides, dipeptides, and terpenoids are the predominant classes comprising these compounds, representing 25%, 25%, 10%, and 10%, respectively. Furthermore, regarding their biological activities, 80% is represented by compounds with anticancer and antimicrobial activities. All compounds contain aromatic rings in their scaffolds, as shown in Supplementary (**Figure 1**).

Additionally, all compounds have -O-, -N-, and/or -NH- groups in their structures. Considering the molecules with the best predicted affinities to *R. microplus* TIM, the MolDock scores for paclitaxel, dirithromycin, toposar, natamycin, and cabazitaxel were -171.258, -168.586, -149.368, -148.880, and -148.810, respectively (**Table 1**). These compounds commonly interact with the residues Tyr67, Val69, Glu70, Gln71, Phe74, and Met82 of TIM (**Figure 1**). The physicochemical characteristics and predicted ADMET properties of these natural products are shown in (**Table 2)**.

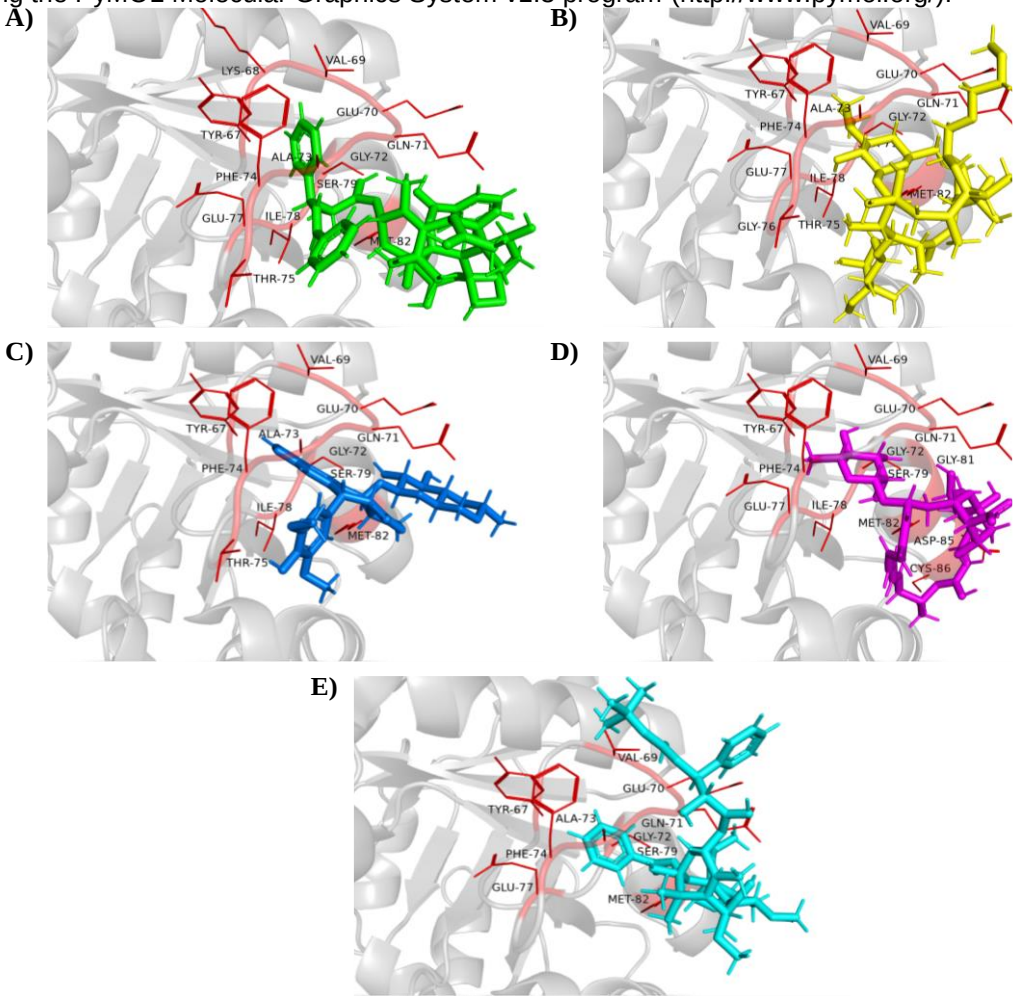
Paclitaxel, dirithromycin, toposar, natamycin, and cabazitaxel present molecular weights of 853.330, 834.550, 588.180 665.300, and 835.380 g/mol, respectively. All compounds have octanol/water partition coefficient (LogP) values below 5.0. Only paclitaxel, toposar, and cabazitaxel are predicted to have high human intestinal absorption (83.692%, 92.199%, and 91.281%, respectively). Natamycin showed positive Ames test mutagenicity. Furthermore, it was predicted that the compounds could enter the brain, as the values for their distribution across the blood-brain barrier (BBB) were 0.024, 0.044, 0.037, 0.045, and 0.023, for paclitaxel, dirithromycin, toposar, natamycin, and cabazitaxel, respectively.

Table 1- Virtual screening results showing the highest ranked compounds based on MolDock score in triosephosphate isomerase of *Rhipicephalus microplus*.

Name	Zinc code	Biological activity	Chemical class	Molecule formula	MolDock score
Paclitaxel	96006020	Anticancer	Diterpenoid	$C_{47}H_{51}NO_{14}$	-171.258
Dirithromycin	96095661	Antibiotic	Aminoglycoside	$C_{42}H_{78}N_2O_{14}$	-168.586
Toposar	3938684	Anticancer	Podophyllotoxin	$C_{29}H_{32}O_{13}$	-149.368
Natamycin	253633751	Antifungal	Aminoglycoside	$C_{33}H_{47}NO_{13}$	-148.880
Cabazitaxel	85536932	Anticancer	Diterpenoid	$C_{45}H_{57}NO_{14}$	-148.810
Dronedarone	49933061	Antiarrhythmic	Aryl-phenylketones	$C_{31}H_{44}N_2O_5S$	-144.797
Cytisine	1599729	Help with smoking cessation	Alkaloid	$C_{11}H_{14}N_2O$	-142.495
Hyperforin	4097413	Antidepressant/Anxiolytic	Bicyclic monoterpenoid	$C_{35}H_{52}O_4$	-141.420
Vinblastine	85555528	Anticancer	Alkaloid	$C_{46}H_{58}N_4O_9$	-140.789
Vindesine	8214470			$C_{43}H_{55}N_5O_7$	-138.474
Synribo	43450324			$C_{29}H_{39}NO_9$	-135.033
Oxacillin	3875439	Antibiotic	Dipeptide	$C_{19}H_{19}N_3O_5S$	-134.530
Docetaxel	85537053	Anticancer	Diterpenoid	$C_{43}H_{53}NO_{14}$	-134.288
Aztreonam	3830264	Antibiotic	Monobactam	$C_{13}H_{17}N_5O_8S_2$	-133.567
Cloxacillin	3875417		Dipeptide	$C_{19}H_{18}ClN_3O_5S$	-131.394
Vincristine	85432549	Anticancer	Alkaloid	$C_{46}H_{56}N_4O_{10}$	-130.217
Paromomycin	60183170	Antibiotic	Aminoglycoside	$C_{23}H_{45}N_5O_{14}$	-129.937
Erythromycin	85534336			$C_{37}H_{67}NO_{13}$	-129.235
Steviolbioside	79216653	Antiviral	Glycoside	$C_{32}H_{50}O_{13}$	-129.203
Streptomycin	8214681	Antibiotic	Aminoglycoside	$C_{21}H_{39}N_7O_{12}$	-129.188

Source: Bezerra WAS, et al., 2024.

Figure 1- Cartoon representation of triosephosphate isomerase (TIM) of *Rhipicephalus microplus* (PDB ID: 3TH6) in complex with A) paclitaxel; B) dirithromycin; C) toposar; D) natamycin; and E) cabazitaxel. The ligands are shown as sticks. In red, TIM residues within 3.5 Å of the natural product. The best pose of TIM with each ligand was visualized and analyzed using the PyMOL Molecular Graphics System v1.3 program (http://www.pymol.org/).



Source: Bezerra WAS, et al., 2024.

Table 2- Physicochemical and ADMET properties predicted for natural compounds.

ID	Paclitaxel	Dirithromycin	Toposar	Natamycin	Cabazitaxel
Molecular weight (g/mol)	853.330	834.550	588.180	665.300	835.380
Hydrogen-bond acceptors	15	16	13	14	15
Hydrogen-bond donors	4	5	3	8	3
LogP	3.580	3.684	1.387	0.091	4.432
Caco2	20.426	41.428	19.140	14.279	23.023
HIA	91.281	66.365	83.692	20.963	92.199
MDCK	0.0434	0.0434	0.0567	0.0437	0.0434
PGP_inh	Non	Inhibitor	Non	Inhibitor	Non
PPB	86.187	14.557	56.392	33.038	82.401
PWS (mg/L)	0.0031	79.197	12.196	52.085	0.0168
Skin_Permeability	-1.834	-3.601	-4.655	-2.770	-1.467
BBB	0.024	0.044	0.037	0.045	0.023
CYP_2C19_inh	Non	Non	Inhibitor	Inhibitor	Non
CYP_2C9_inh	Inhibitor	Non	Inhibitor	Inhibitor	Non
CYP_2D6_inh	Non	Inhibitor	Non	Inhibitor	Non
CYP_2D6_sub	Non	Weakly	Non	Non	Non
CYP_3A4_inh	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor
CYP_3A4_sub	Substrate	Substrate	Substrate	Weakly	Substrate
Algae_at	0.00039	0.00031	0.01727	0.00354	0.00054
Ames_test	Non-mutagen	Non-mutagen	Non-mutagen	Mutagen	Non-mutagen
Carcino_Mo	Positive	Negative	Negative	Positive	Negative
Carcino_Rat	Negative	Negative	Negative	Positive	Negative
daphnia_at	0.01371	0.10608	0.29523	0.92061	0.01212
hERG_inh	Ambiguous	Ambiguous	Ambiguous	Ambiguous	Low_risk
Medaka_at	0.0007	0.0277	0.1803	1.769	0.0005
Minnow_at	0.0051	0.0303	0.2889	3.478	0.0029

Note: BBB – Blood-Brain Barrier (C.brain/C.blood); Caco-2 – Caco2-cell model; HIA – Human Intestinal Absorption model (HIA, %); MDCK – Madin-Darby Canine Kidney (nm/sec); PGP_inhibition – P-glycoprotein inhibitor; PPB – Plasma Protein Binding (%); PWS – Pure water solubility (mg/L); Skin Permeability- Skin permeability in cm/hour. Algae at – algae test (mg/L); Ames Test – Ames Salmonella; CYP – Cytochrome P450; Carcino M – carcinogenesis test in the mouse; Carcino R – carcinogenesis test in rats; Daphnia at – test on crustacean Daphnia; hERG inhib. – hERG-controlled potassium channel inhibition; Medaka_at – test on medaka fish; Minnow_at – test on small freshwater fish.
Source: Bezerra WAS, et al., 2024.

DISCUSSION

Synthetic acaricides are extensively utilized in veterinary and human medicine for parasitic disease control. However, the emergence of resistance underscores the need for alternative approaches (OBAID MK, et al., 2022). Exploration of new bioactive compounds with enhanced potency and selectivity for tick targets offers potential solutions to these challenges. In silico methods such as molecular docking facilitate the discovery of novel compounds that bind to parasites' molecular targets (RUYCK J, et al., 2016).

Triosephosphate isomerase (TIM) is an enzyme involved in both glycolysis and gluconeogenesis where it catalyzes the interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. To produce selective inhibitors, it appeared to be essential to target the enzyme's dimer interface, which exhibits poor conservation between species, in contrast to the enzyme's overall structure which is well conserved (TÉLLEZ-VALENCIA A, et al., 2004).

In this study, molecular docking of natural products from the ZINC15 database onto the *R. microplus* TIM protein was conducted. Docking analysis predicts the optimal molecular orientation for binding of a compound to a protein and calculates the corresponding binding energy (MALAK N, et al., 2023). The 20 compounds with the best MolDock scores (**Table 1**), including paclitaxel, dirithromycin, toposar, natamycin, and cabazitaxel, were suggested as potential TIM ligands targeting the protein's dimer interface what is expected to lead to inhibition since a dimeric structure is required for activity (NÁJERA H, et al., 2003).

Although these compounds have antiprotozoal or antimicrobial activity (BENAIM G, et al., 2014; PENSEL PE, et al., 2014; AWASTHI BP, MITRA K, 2018; PICHKUR EB, et al., 2020; CAO Y, et al., 2023), to the best of our knowledge, none has yet been described in the literature as having activity against ticks. However,

alkaloids and terpenes, two of the classes to which compounds belong that were identified in the present study, have been reported to possess anti-tick activity (CARROLL JF, et al., 2007; LIMA HG, et al., 2020; CARDOSO AS, et al., 2020; SILVA GD, et al., 2021).

In medicinal chemistry, similar molecules exhibit similar biological effects, guiding the modification of active compounds. Bioisosteric replacements transform lead structures into enzyme inhibitors, receptor agonists/antagonists, and other active agents. This systematic replacement strategy enables to optimize drug properties, yielding a variety of therapeutically effective medications (KUBINYI H, 2002; POUPAERT J, et al., 2005).

However, it needs to be noted that several surprising structure-activity relationships demonstrate that chemically similar compounds may exhibit significantly different biological actions and activities (DINARVAND M, SPAIN M, 2021; ALIZADEH SR, EBRAHIMZADEH MA, 2022; EBETINO FH, et al., 2022; SHAMSUDIN NF, et al., 2022). It is important to highlight that the structures identified in the present study have functional groups in common that could be explored in the early stages of drug design to develop novel TIM inhibitors. As shown in **Supplementary Figure 1**, all compounds have -O-, and/or -N- / -NH- groups.

The selected compounds' significant delocalized conjugated structures play a pivotal role in exerting pharmacological activity (KAUR R, KUMAR K, 2021; LIU R, et al., 2022). Moreover, interactions between aromatic and heteroaromatic rings are major contributors to protein structure and protein–ligand complexation (FASAN R, et al., 2006; SALONEN LM, et al., 2011). In this study, the 20 selected compounds contain aromatic rings. Previous reports have shown TIM inhibitors that bind to the interface through aromatic interactions (JUÁREZ-SALDIVAR A, et al., 2021; KURKCUOGLU Z, et al., 2015).

In this study, TIM residues within a proximity of 3.5 Å to the compounds were evaluated, a distance suggested as ideal for interaction (LIU Z, et al., 2008; BIANCHI V, et al., 2012). The residues Tyr67, Val69, Glu70, Gln71, Gly72, Phe74, Ser79, and Met82 in *R. microplus* TIM may be key residues involved in the interaction, as they are all within 3.5 Å of each of the five selected compounds (Figure 1). These compounds—paclitaxel, dirithromycin, toposar, natamycin, and cabazitaxel—bind to the TIM dimer interface at residues in Loop 3 (**Figure 1**).

Considering that Loop 3 residues are involved in hydrogen-bond interactions with Loop 1 of the other subunit, contributing to the integrity of the dimer (SARAMAGO L, et al., 2018), it is suggested that these compounds could act by perturbing the interface region, leading to dimer rupture, similar as reported for other TIM inhibitors (OLIVARES-ILLANA V, et al., 2007; OLIVER C, TIMSON DJ, 2017; SARAMAGO L, et al., 2018; VÁZQUEZ-JIMÉNEZ LK, et al., 2022).

Saramago et al. (2018) demonstrated that a compound from the benzofuroxan family, with an IC_{50} on *R. microplus* TIM of 49 µM, binds to the dimer interface, interacting with residues on Loop 3 (Glu70, Gln71, Ser79, and Met82), as observed for the interaction of the aforementioned compounds (**Figure 1**). Although TIM is also an essential enzyme in mammals, only natural products approved for human use were selected to minimize the risk of mammalian toxicity. The physicochemical parameters and predicted ADMET properties of natural products were analyzed using the PreADMET tool (**Table 2**).

None of the compounds comply with Lipinski's rule of five, a guiding principle for assessing drug likeness and designing chemical compounds for potential oral activity (LIPINSKI CA, et al., 1997; RAJALAKSHMI R, et al., 2021). However, it is worth noting that many natural products which don't comply with Lipinski's rule criteria are still capable of traversing cell membranes (LEESON PD, DAVIS AM, 2004; O'SHEA R, MOSER HE, 2008; ABDELMOHSEN UR, et al., 2017).

For instance, the functional mechanisms of paclitaxel primarily involve inhibiting the dynamics of the microtubule spindle, thereby controlling cell proliferation and DNA repair (KHANNA C, et al., 2015; YAN-HUA Y, et al., 2020). Based on the findings of this study, the investigated natural products may serve as potential inhibitors of the TIM enzyme in *R. microplus* or as lead compounds for the design of new inhibitors. Expanding research into TIM inhibition and further modifying or synthesizing compounds based on these scaffolds could enhance their efficacy, potentially leading to their application as acaricides. This could significantly contribute to the development of innovative anti-tick drugs.

CONCLUSION

In this study, virtual screening based on molecular docking was employed to identify natural compounds among FDA-approved drugs that are predicted to bind to TIM from *R. microplus* and interfere with its activity. These findings offer promise for the development of novel strategies to combat acaricide-resistant cattle ectoparasites, potentially mitigating economic losses in the livestock industry.

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REFERENCES

1. ABDELMOHSEN UR, et al. Potential of marine natural products against drug-resistant fungal, viral, and parasitic infections. The Lancet Infectious Diseases. 2017; 17(2): 30-41.
2. AGWUNOBI DO, et al. A retrospective review on ixodid tick resistance against synthetic acaricides: implications and perspectives for future resistance prevention and mitigation. Pesticide Biochemistry Physiology. 2021; 173: 1-17.
3. ALIZADEH SR, EBRAHIMZADEH MA. O-Glycoside quercetin derivatives: Biological activities, mechanisms of action, and structure–activity relationship for drug design, a review. Phytotherapy Research. 2022; 36(2): 778-807.
4. AWASTHI BP, MITRA K. In vitro leishmanicidal effects of the anti-fungal drug natamycin are mediated through disruption of calcium homeostasis and mitochondrial dysfunction. Apoptosis. 2018;23(7): 420-35.
5. BENAIM G, et al. Dronedarone, an amiodarone analog with improved anti-Leishmania mexicana efficacy. Antimicrobial agents chemotherapy. 2014; 58(4): 2295-303.
6. BEZERRA WAS, et al. Anonaine from Annona crassiflora inhibits glutathione S-transferase and improves cypermethrin activity on Rhipicephalus (Boophilus) microplus (Canestrini, 1887). Experimental Parasitology. 2022; 243: 1-8.
7. BIANCHI V, et al. Identification of binding pockets in protein structures using a knowledge-based potential derived from local structural similarities. BioMed Central Bioinformatics. 2012; 13: 1-13.
8. BRAZ V, et al. Inhibition of energy metabolism by 3-bromopyruvate in the hard tick Rhipicephalus microplus. Comparative Biochemistry Physiology Part C: Toxicology Pharmacology. 2019; 218: 55-61.
9. CAO Y, et al. Study on the Antifungal Activity and Potential Mechanism of Natamycin against Colletotrichum fructicola. Journal of Agricultural Food Chemistry. 2023; 71(46): 17713-22.
10. CARDOSO AS, et al. Terpenes on Rhipicephalus (Boophilus) microplus: Acaricidal activity and acetylcholinesterase inhibition. Veterinary parasitology. 2020; 280: 1-5.
11. CARROLL JF, et al. Repellency of two terpenoid compounds isolated from Callicarpa americana (Lamiaceae) against Ixodes scapularis and Amblyomma americanum ticks. Experimental applied acarology. 2007; 41: 215-24.
12. CHOUBEY SK, JEYARAMAN J. A mechanistic approach to explore novel HDAC1 inhibitor using pharmacophore modeling, 3D-QSAR analysis, molecular docking, density functional and molecular dynamics simulation study. Journal of Molecular Graphics Modelling. 2016; 70: 54-69.
13. DINARVAND M, SPAIN M. Identification of bioactive compounds from marine natural products and exploration of Structure-Activity Relationships (SAR). Antibiotics. 2021; 10(3): 1-24.
14. EBETINO FH, et al. Bisphosphonates: The role of chemistry in understanding their biological actions and structure-activity relationships, and new directions for their therapeutic use. Bone. 2022; 156: 116289.
15. FASAN R, et al. Structure–activity studies in a family of β-Hairpin protein epitope mimetic inhibitors of the p53–HDM2 protein–protein interaction. Chemistry biology Chemistry. 2006; 7(3): 515-26.
16. GANESAN M, et al. Design, synthesis, α-amylase inhibition and in silico docking study of novel quinoline bearing proline derivatives. Journal of Molecular Structure. 2020; 1208: 1-51.

17. GONZÁLEZ-MORALES LD, et al. Triose phosphate isomerase structure-based virtual screening and in vitro biological activity of natural products as Leishmania mexicana inhibitors. Pharmaceutics. 2023; 15(8): 1-17.
18. GRISI L, et al. Reassessment of the potential economic impact of cattle parasites in Brazil. Revista Brasileira de Parasitologia Veterinária. 2014; 23: 150-6.
19. JONSSON N. The productivity effects of cattle tick (Boophilus microplus) infestation on cattle, with particular reference to Bos indicus cattle and their crosses. Veterinary parasitology. 2006; 137(1-2): 1-10.
20. JUÁREZ-SALDIVAR A, et al. Virtual screening of fda-approved drugs against triose phosphate isomerase from Entamoeba histolytica and Giardia lamblia identifies inhibitors of their trophozoite growth phase. International Journal of Molecular Sciences. 2021; 22(11): 1-8.
21. KAUR R, KUMAR K. Synthetic and medicinal perspective of quinolines as antiviral agents. European Journal of Medicinal Chemistry. 2021; 215: 1-39.
22. KHANNA C, et al. A review of paclitaxel and novel formulations including those suitable for use in dogs. Journal of veterinary internal medicine. 2015; 29(4): 1006-12.
23. KIAMETIS AS, et al. Potential acetylcholinesterase inhibitors: molecular docking, molecular dynamics, and in silico prediction. Journal of molecular modeling. 2017; 23: 1-1.
24. KLAFKE G, et al. Multiple resistance to acaricides in field populations of Rhipicephalus microplus from Rio Grande do Sul state, Southern Brazil. Ticks tick-borne diseases. 2017; 8(1): 73-80.
25. KUBINYI H. Chemical similarity and biological activities. Journal of the Brazilian Chemical Society. 2002; 13(6): 717-26.
26. KUMAR K, et al. Cloning, overexpression and characterization of Leishmania donovani triosephosphate isomerase. Experimental parasitology. 2012; 130(4): 430-6.
27. KURKCUOGLU Z, et al. How an inhibitor bound to subunit interface alters triosephosphate isomerase dynamics. Biophysical Journal. 2015; 109(6): 1169-78.
28. KWANG LS. Editor In silico high-throughput screening for ADME/Tox properties: PreADMET program. Abstr Conf Comb Chem Jpn, 2005.
29. LEESON PD, DAVIS AM. Time-related differences in the physical property profiles of oral drugs. Journal of medicinal chemistry. 2004; 47(25): 6338-48.
30. LIANG N, et al. Characterization and evaluation of a new triosephosphate isomerase homologue from Haemaphysalis longicornis as a candidate vaccine against tick infection. Ticks Tick-borne Diseases. 2022; 13(4): 1-7.
31. LIMA HG, et al. Anti-tick effect and cholinesterase inhibition caused by Prosopis juliflora alkaloids: in vitro and in silico studies. Revista Brasileira de Parasitologia Veterinária. 2020; 29: 1-15.
32. LIPINSKI CA, et al. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced drug delivery reviews. 1997; 23(1-3): 3-25.
33. LIU R, et al. Research progress on the biological activities of metal complexes bearing polycyclic aromatic hydrazones. Molecules. 2022; 27(23): 1-56.
34. LIU Z, et al. Geometrical Preferences of the Hydrogen Bonds on Protein− Ligand Binding Interface Derived from Statistical Surveys and Quantum Mechanics Calculations. Journal of Chemical Theory Computation. 2008; 4(11): 1959-73.
35. MALAK N, et al. Density Functional Theory Calculations and Molecular Docking Analyses of Flavonoids for Their Possible Application against the Acetylcholinesterase and Triose-Phosphate Isomerase Proteins of Rhipicephalus microplus. Molecules. 2023; 28(8): 1-20.
36. MARQUES R, et al. Climate change implications for the distribution of the babesiosis and anaplasmosis tick vector, Rhipicephalus (Boophilus) microplus. Veterinary research. 2020; 51: 1-10.
37. MIRABALLES C, et al. Rhipicephalus microplus, babesiosis and anaplasmosis in Uruguay: current situation and control or elimination programs on farms. Experimental Applied Acarology. 2019; 78: 579-93.
38. NÁJERA H, et al. Thermodynamic characterization of yeast triosephosphate isomerase refolding: insights into the interplay between function and stability as reasons for the oligomeric nature of the enzyme. Biochemical Journal. 2003; 370(3): 785-92.
39. NICARETTA JE, et al. Selective versus strategic control against Rhipicephalus microplus in cattle: A comparative analysis of efficacy, animal health, productivity, cost, and resistance management. Veterinary Parasitology. 2023; 321: 1-16.
40. O'SHEA R, MOSER HE. Physicochemical properties of antibacterial compounds: implications for drug discovery. Journal of medicinal chemistry. 2008; 51(10): 2871-8.
41. OBAID MK, et al. Acaricides resistance in ticks: selection, diagnosis, mechanisms, and mitigation. Frontiers in Cellular Infection Microbiology. 2022; 12: 1-20.

42. OLIVARES-ILLANA V, et al. Perturbation of the dimer interface of triosephosphate isomerase and its effect on Trypanosoma cruzi. PLoS neglected tropical diseases. 2007; 1(1): 1-8.
43. OLIVER C, TIMSON DJ. In silico prediction of the effects of mutations in the human triose phosphate isomerase gene: towards a predictive framework for TPI deficiency. European Journal of Medical Genetics. 2017; 60(6): 289-98.
44. PENSEL PE, et al. In vitro effect of 5-fluorouracil and paclitaxel on Echinococcus granulosus larvae and cells. Acta tropica. 2014; 140: 1-9.
45. PICHKUR EB, et al. Insights into the improved macrolide inhibitory activity from the high-resolution cryo-EM structure of dirithromycin bound to the E. coli 70S ribosome. RNA. 2020; 26(6): 715-23.
46. POUPAERT J, et al. 2 (3H)-benzoxazolone and bioisosters as “privileged scaffold” in the design of pharmacological probes. Current medicinal chemistry. 2005; 12(7): 877-85.
47. RAJALAKSHMI R, et al. In silico studies: Physicochemical properties, drug score, toxicity predictions and molecular docking of organosulphur compounds against Diabetes mellitus. Journal of Molecular Recognition. 2021; 34(11): 1-21.
48. RUYCK J, et al. Molecular docking as a popular tool in drug design, an in silico travel. Advances Applications in Bioinformatics Chemistry. 2016: 1-11.
49. SALONEN LM, et al. Aromatic rings in chemical and biological recognition: energetics and structures. Angewandte Chemie International Edition. 2011; 50(21): 4808-42.
50. SAPORITI T, et al. Phenotypic and Target-Directed Screening Yields New Acaricidal Alternatives for the Control of Ticks. Molecules. 2022; 27(24): 1-14.
51. SARAMAGO L, et al. Novel and selective Rhipicephalus microplus triosephosphate isomerase inhibitors with acaricidal activity. Veterinary Sciences. 2018; 5(3): 1-19.
52. SELLES SMA, et al. Acaricidal and repellent effects of essential oils against ticks: a review. Pathogens. 2021; 10(11): 1-17.
53. SHAMSUDIN NF, et al. Antibacterial effects of flavonoids and their structure-activity relationship study: A comparative interpretation. Molecules. 2022; 27(4): 1-43.
54. SILVA GD, et al. In vitro and in silico studies of the larvicidal and anticholinesterase activities of berberine and piperine alkaloids on Rhipicephalus microplus. Ticks Tick-borne Diseases. 2021; 12(2): 1-6.
55. TÉLLEZ-VALENCIA A, et al. Inactivation of triosephosphate isomerase from Trypanosoma cruzi by an agent that perturbs its dimer interface. Journal of molecular biology. 2004; 341(5): 1355-65.
56. VÁZQUEZ-JIMÉNEZ LK, et al. Ligand-based virtual screening and molecular docking of benzimidazoles as potential inhibitors of triosephosphate isomerase identified new trypanocidal agents. International Journal of Molecular Sciences. 2022; 23(17): 1-27.
57. WADOOD A, et al. In-silico drug design: An approach which revolutionarised the drug discovery process. Drug design and delivery. 2013;1(1):1-4.
58. WALDMAN J, et al. Putative target sites in synganglion for novel ixodid tick control strategies. Ticks Tick-borne Diseases. 2023; 14(3): 1-12.
59. YAN-HUA Y, et al. Research progress on the source, production, and anti-cancer mechanisms of paclitaxel. Chinese journal of natural medicines. 2020; 18(12): 890-7.