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Docking molecular do complexo de rutênio com epiisopiloturina e óxido nítrico frente à proteína nucleoside diphosphate kinase da Leishmania

Molecular docking of rutenum complex with epiisopyloturin and nitric oxide against nucleoside diphosphate kinase protein Leishmania

Acoplamiento molecular del complejo de rutenio con epiisopiloturina y óxido nítrico contra la proteína de nucleoside diphosphate kinase de Leishmania

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Resumo

A leishmaniose é uma doença infecciosa que afeta animais e humanos, causada por parasitas flagelados pertencentes ao gênero Leishmania, podendo apresentar-se em diferentes formas clínicas, dependendo da cepa infectante e da reação imune do hospedeiro. Estima-se que a doença atinja cerca de 700.000 a 1 milhão de pessoas, causando a morte de 20 a 30.000 indivíduos anualmente. Assim, o presente estudo tem como objetivo realizar uma simulação de acoplamento molecular do complexo de rutênio com epiisopiloturina e óxido nítrico contra a proteína Nucleosídeo difosfato cinase de *Leishmania amazonensis*. A molécula em 3D NDK foi extraída do banco de dados de proteínas e ácidos nucleicos PDB. A estrutura molecular 3D do complexo Epiruno$_2$ foi projetada usando o *software gaussview 5.0*. O alvo NDK e o complexo Epiruno$_2$ foram preparados para simulações de ancoragem, onde o NDK foi considerado rígido e o Epiruno$_2$ foi considerado flexível. O complexo Epiruno$_2$ apresentou boa taxa de afinidade molecular com a proteína alvo, tornando-o atrativo para ensaios experimentais em laboratórios para a proteína NDK da Leishmania e NDKs de outros patógenos, no entanto, o fármaco miltefosina apresentou baixa afinidade molecular para o mesmo alvo, corroborando com estudos apresentados na literatura sobre a eficácia reduzida dos medicamentos atuais contra a leishmaniose.

Palavras-chave: Biologia Computacional; Anti-Infecciosos; Trypanosomatina.

Abstract

Leishmaniasis is an infectious disease that affects both animals and humans, caused by flagellated parasites belonging to the genus Leishmania may present in different clinical forms depending on the infecting strain and the immune reaction of the host. The disease is estimated to reach about 700,000 to 1 million people, causing the deaths of 20 to 30,000 individuals annually. Thus, the present study aims to perform a molecular coupling simulation of the ruthenium complex with epiisopiloturin and nitric oxide against the protein Nucleoside diphosphate kinase from *Leishmania amazonensis*. The NDK 3D molecule was extracted from the PDB nucleic proteins and acids database. The 3D molecular structure of the Epiruno$_2$ complex was designed using *gaussview 5.0 software*. The NDK target and Epiruno$_2$ complex were prepared for docking simulations, where NDK was considered rigid and

Epiruno$_2$ was considered flexible. The Epiruno$_2$ complex presented a good molecular affinity rate with the target protein, making it attractive for experimental trials in laboratories for Leishmania's NDK protein and NDKs of other pathogens, however, the drug miltefosin presented low molecular affinity for the same target, corroborating studies presented in the literature on the reduced efficacy of current drugs against leishmaniosis.

Keywords: Computational Biology; Anti-Infective Agents; Trypanosomatina.

Resumen

La leishmaniasis es una enfermedad infecciosa que afecta a animales y humanos, causada por parásitos flagelados pertenecientes al género Leishmania, y puede presentarse en diferentes formas clínicas, dependiendo de la cepa infectante y la reacción inmune del huésped. Se estima que la enfermedad afecta a alrededor de 700,000 a 1 millón de personas, causando la muerte de 20 a 30,000 personas anualmente. Por lo tanto, el presente estudio tiene como objetivo realizar una simulación de acoplamiento molecular del complejo de rutenio con epiisopiloturina y óxido nítrico contra la proteína nucleósido difosfato quinasa de *Leishmania amazonensis*. La molécula 3D NDK se extrajo de la base de datos de proteínas y ácidos nucleicos PDB. La estructura molecular 3D del complejo Epiruno$_2$ se diseñó utilizando el software gaussview 5.0. El proteína NDK y el complejo Epiruno$_2$ fueron preparados para simulaciones de anclaje, donde NDK se consideró rígido y Epiruno$_2$ se consideró flexible. El complejo Epiruno$_2$ tenía una buena tasa de afinidad molecular con la proteína, lo que lo hacía atractivo para las pruebas experimentales de laboratorio para la proteína Leishmania NDK y NDK de otros patógenos; sin embargo, el fármaco miltefosina mostró baja afinidad molecular con el mismo proteína, corroborando los estudios en la literatura sobre la reducción de la eficacia de los medicamentos contra la leishmaniasis actuales.

Palabras clave: Biología Computacional; Antiinfecciosos; Trypanosomatina.

1. Introduction

Leishmaniasis is an infectious disease that affects both animals and humans, caused by flagellated parasites belonging to the genus Leishmania may present in different clinical forms depending on the infecting strain and the host immune reaction. It is estimated that the disease affects about 700,000 to 1 million people, causing the deaths of 20 to 30,000 individuals annually (WHO, 2019). This disease is present in more than 98 countries in the

world, especially in regions with tropical climate and poor sanitation, and is therefore considered a disease associated with poverty.

Thus, pharmaceutical companies have no interest in developing new drugs as treatment alternatives, even if the number of new cases is exorbitant, which would generate market demand, however, because it is a disease that affects the poorer regions. In other words, without economy power, there is a lack of interest in the sector, which would spend billions on investments in the discovery of new drugs without a prospect of financial return to industries (BOUCHER et al., 2009; THEURETZBACHER, 2009).

Thus, people diagnosed with this disease undergo current treatments available, where the drugs have reduced efficacy and cause various side effects to patients, who in many cases prefer not to undergo treatment so that they do not feel the side effects caused by the drugs. The main drugs to combat Leishmania are: N-methylglucamine antimonial; amphotericin B; and miltefosine (KHAMESIPOUR, 2014).

This reduced efficacy of current pharmaceutical drugs is of concern, as there are numerous strains that have acquired resistance to almost all pharmacological agents available on the market, which explicitly requires the production of new substances with good pharmacological activity and new mechanisms of action (Kasbekar, 2006).

Faced with this problem, the search for new drugs for the treatment of leishmaniasis is fundamental to combat resistant strains of the various species of the genus Leishmania, where the substance has lower toxicity and high efficacy. In this sense, it has been shown that metal complexes coupled to a naturally occurring bioactive ligand present potential activity against different parasites, such as Leishmania (GARBIN et al., 2015). This strategy is achieved by combining a bioactive agent with a metal fragment that results in a single molecule that can translate into enhanced activity against parasites (SÁNCHEZ-DELGADO et al., 1998; MACEDO et al., 2017).

In this sense, the ruthenium complex with epiisopiloturin and nitric oxide ($Epiruno_2$) showed antiparasitic activity, in studies by Rocha et al. (2018) who analyzed by computational quantum chemistry and molecular biology the interaction between the $Epiruno_2$ complex and *Schistosoma mansoni* (*S. mansoni*) targets, where the complex showed high molecular affinity rates with targets and high rate of inhibition constants. This antiparasitic study against *S. mansoni* targets may indicate that the $Epiruno_2$ complex also has molecular affinity for Leishmania targets.

Thus, the present study aims to perform a molecular coupling simulation of the ruthenium complex with epiisopiloturin and nitric oxide against the protein Nucleoside

diphosphate kinase from *Leishmania amazonensis*.

2. Materials and Method

The Nucleoside diphosphate kinase (NDK) 3D molecule was obtained from the *Protein Data Bank* (PDB) database with the identification code 5go1 (BERMAN et al., 2000). The 3D molecular structure of the Epiruno$_2$ complex was designed using *GaussView 5.0 software* (DENNINGTON et al., 2009) and optimized by DFT (Density Functional Theory) calculation using the B3lyp functional and the 6-311^{++}G(d, p) base set available from *Gaussian 09W software* (KOHN & SHAM, 1965; FRISCH et al., 2009).

The molecular coupling simulations followed the protocol developed by Rocha et al. (2018) with some modifications. All coupling procedures used *AutoDockTools-1.5.6 software* (GOODSELL et al., 1996). The NDK target and Epiruno$_2$ complex were prepared for coupling simulations, where NDK was considered rigid and Epiruno$_2$ was considered flexible. Partial charges were calculated after the addition of all hydrogens. The nonpolar hydrogen atoms of the protein and binder were subsequently fused. A 60 × 60 × 60 point cubic box with a spacing of 0.375 Å between grid points was generated for the simulations. The affinity grid center was defined at the NDK receptor active site residue. The Lamarckian global search (LGA) genetic algorithm (MORRIS et al., 1998) and the pseudo-Solis and Wets (SOLIS & WETS, 1981) local search (LS) methods were applied in the search for molecular anchor. The Epiruno$_2$ complex was subjected to 100 independent runs of coupling simulations (RAMOS et al., 2012). The other docking parameters were set to default values. The same protocol was performed for the drug miltefosine used as a control in this study for comparison with the Epiruno$_2$ complex.

The criteria adopted for the analyzes were defined by the results presented with lower G_{bind}^{a} energy with visual inspection, in addition to the hydrogen bond interactions and inhibition constants presented by the Epiruno$_2$ complex and the drug miltefosine.

3. Results and discussion

Molecular coupling between the Epiruno$_2$ complex and *L. amazonensis* NDK protein showed promising G_{bind}^{a} energy molecular affinity results of -7.59 Kcal.mol^{-1} and an inhibition constant of 2.7 µM (Table 01). These results were superior compared to the results presented by the drug miltefosine against the same target of *L. amazonensis*, with G_{bind}^{a}

energy of -2.7 Kcal.mol^{-1} and an inhibition constant of 10.57 mM (Table 01). This preliminary Docking analysis indicates the low molecular affinity of the drug miltefosine in interacting with the *L. amazonensis* NDK protein at its active site (MENG et al., 2011). This shows the reduced efficacy of the drug, as parasitic strains are increasingly resistant to current drugs available from the pharmaceutical industries, as these pharmacological residues take time to be excreted by patients and parasites develop defense mechanisms against these drugs, acting on drug inhibition (ROJAS et al., 2006).

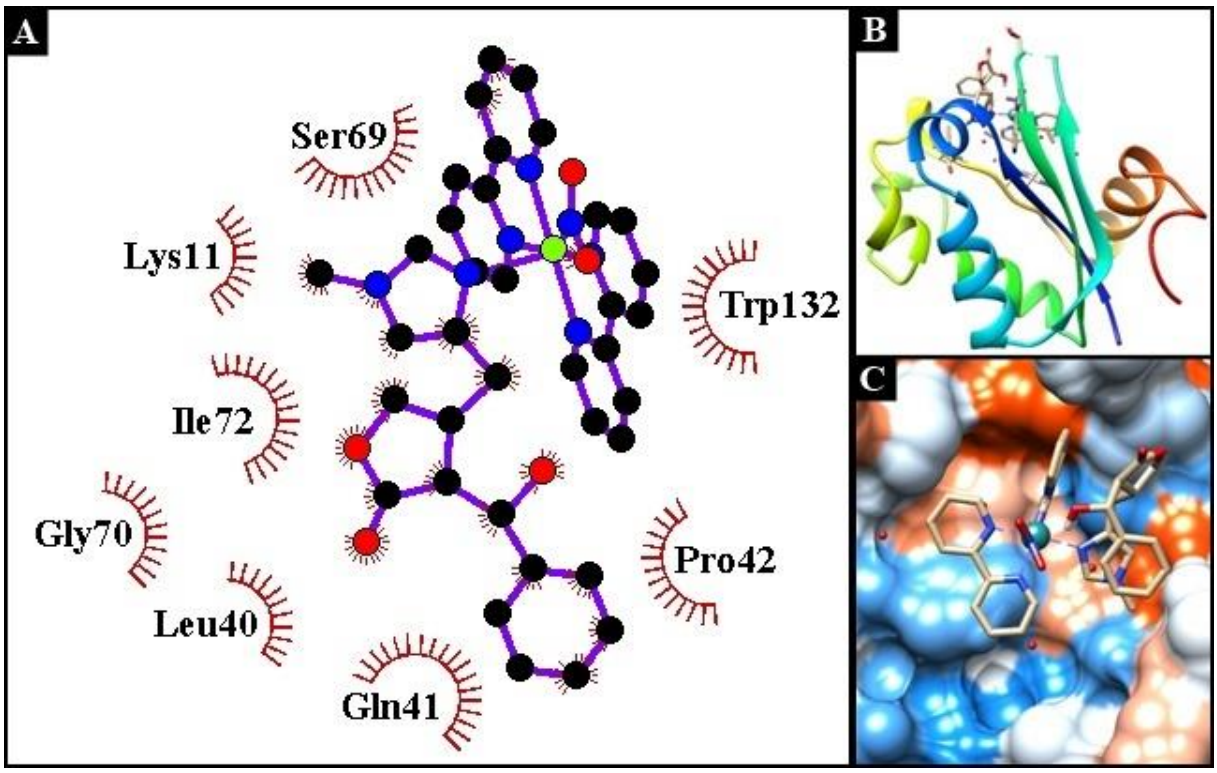
Table 01: Molecular affinity parameters of Epiruno$_2$ complex and miltefosine drug against *L. amazonensis* NDK protein.

Complex (protein binder)	**ΔG_{bind} [a] (kcal mol^{-1})**	**Ki[b] (µM)**	**Number of Independent Snap Races**	**Number of Conformations in First Cluster**	**Interacting Amino Acids Through Hydrogen Bonds**
Epiruno$_2$/NDK	-7.59	2,7 µM	100	9	-
Miltefosine/NDK	-2,7	10,57 mM	100	5	Met111, Val115

Source: own author (2019).

The molecular interactions of the Epiruno$_2$ complex act directly at the edges of the NDK protein binding site on residues Ser69, Lys11, Ile72, Gly70, Leu40, Gln41, Pro42 and Trp132 which are the hydrophobic bonds formed in the molecular coupling simulation (Figure 01). These results indicate that the complex is attractive for experimental testing in laboratories because the complex binds to the target protein very easily, thereby inhibiting a key protein of this pathogen (COSCONATI et al., 2010; PERRYMAN et al. al., 2015), as it is a ubiquitous and conserved protein that plays a central role in maintaining intracellular NTP levels, which catalyzes the transfer of the γ-phosphate group from NTP to NDP (PARKS et al., 1973). In addition to these functions, this protein has been reported in the literature to be involved in cell development, differentiation, proliferation, apoptosis, tumor metastasis and motility (MACDONALD et al., 1993; KANTOR et al., 1993; LACOMBE et al., 2000).

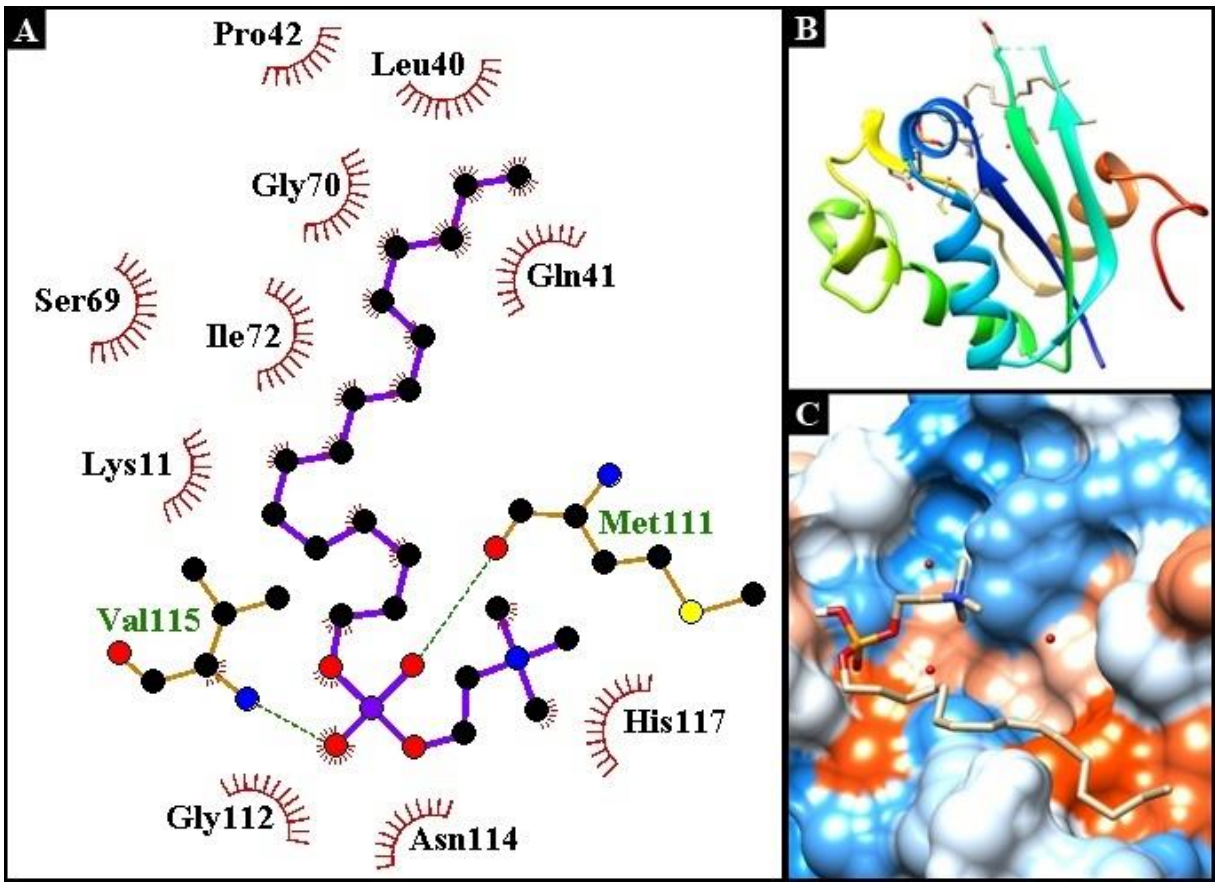
Figure 01: Molecular docking between the Epiruno$_2$ complex and the NDK protein.



A) Molecular interaction between Epiruno$_2$ and the NDK target protein; B) Complex generated after molecular docking; C) Snap to the active site of the NDK target protein.

The molecular interactions of the meiltefosine drug with the *L. amazonensis* NDK target protein formed 2 hydrogen bridges at amino acids Val115 and Met111 which are the most intense bonds performed by the drug and protein (Figure 02). The most attractive binding site for drug-to-target binding was amino acid Lys11 with the coordinates: X = -45,549; Y = 49,719; and Z = 6,909, thus, the drug had a high G_{bind}^{a} energy at the best snap fit orientation demonstrating not having good molecular affinity for the Leishmania target (MENG et al., 2011). This shows the need to look for new treatment alternatives against leishmaniasis, which have new mechanisms of action, high efficacy and low toxicity (KASBEKAR, 2006).

Figure 02: Molecular Docking Between Miltefosine Drug and NDK Protein.



A) Molecular interaction between miltefosine drug and NDK target protein; B) Complex generated after molecular docking; C) Snap to the active site of the NDK target protein.

Associated with this, we also observed that the Epiruno$_2$ complex is an attractive candidate in NDK protein testing of other pathogens, as crystal structures of NDKs have been elucidated from organisms such as: *Drosophila*; *Dictyostelium*; *Myxococcus*; Human; *E. coli*; *S. aureus*; and *L. major* (MISHRA et al., 2017) and through X-ray crystallographic studies of human bacterial NDKs, it has been shown that all NDK proteins have similar subunits of about 150 residues with a fold similar to ferredoxin (βαββαβ). In addition all NDKs have an oligomeric structure where dimer units are assembled into longer multimers, allowing their active site to be conserved from eukaryote prokaryotes (SRIVASTAVA et al., 2011) enabling studies of molecular docking of the Epiruno$_2$ complex for several pathogens.

4. Conclusion

This study concludes via molecular coupling tests the reduced efficacy of miltefosine against Leishmania, showing low molecular affinity for the *L. amazonensis* NDK target protein. However, the Epiruno$_2$ complex showed good molecular affinity against the same

target, with G_{bind}^{a} energy of -7.59 Kcal.mol^{-1} indicating inhibitory action on the active site of a key protein in parasite development, making it attractive for assays experimental laboratory tests as *in vitro*, *in vivo*, *ex vivo* tests against leishmania NDK protein and other pathogenic NDKs.

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