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Avaliação in silico do efeito inibitório dos antirretrovirais Atazanavir e Darunavir sobre a principal protease do SARS-CoV-2: estudos de docking e dinâmica molecular

In silico evaluation of the inhibitory effect of antiretrovirals Atazanavir and Darunavir on the main protease of SARS-CoV-2: docking studies and molecular dynamics

Evaluación in silico del efecto inhibitorio de los antirretrovirales Atazanavir y Darunavir sobre la proteasa principal del SARS-CoV-2: estudios de acoplamiento y dinámica molecular

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Resumo

O SARS-CoV-2 faz parte de uma família vírus de RNA novamente descrito em 2019, sendo o causador da doença Covid-19. A integração de estratégias computacionais tem grande importância na identificação e desenvolvimento de novos compostos promissores. Atazanavir e Darunavir, foram projetados para combater a resistência a fármacos mutantes principalmente por meio do aumento do número de interações polares com os principais átomos da cadeia da protease do HIV. Este estudo visa avaliar a interação molecular dos fármacos Atazanavir e Darunavir com a principal protease do SARS-CoV-2 através de estudos de *docking* e dinâmica molecular. Trata-se de um estudo descritivo, do tipo experimental com uma abordagem quali-quantitativa sobre o tema. Para tanto utilizando-se dos programas BIOVIA Discovery Studio, PyMol, AutoDock Tools 1.5.6, AutoDock Vina, foram realizadas a modelagem e simulação de ancoragem do fármaco no local de ação. Foram demonstradas pontuações menores, sendo -7.0 (Darunavir) a mais próxima do Inibidor UAW 247. É possível perceber que os fármacos demonstraram ligações residuais semelhantes, também, em relação à estrutura da protease, a molécula testada mais próxima foi o Atazanavir. Levando em consideração a estabilidade dos valores RMSD, é válido inferir que em relação ao inibidor UAW 247, o fármaco Atazanavir é aquele que melhor se assemelha, ao contrário do Darunavir, que apresenta variações maiores. Os dois fármacos encaixaram no local de ligação devido principalmente, a interações eletrostáticas e pontes de hidrogênio. O Atazanavir é o que mais se assemelha à atividade molecular, e o Darunavir é o que apresenta melhor pontuação de ancoragem.

Palavras-chave: Ancoragem molecular; Inibidor da protease; Uso de medicamentos.

Abstract

SARS-CoV-2 is part of an RNA virus family described again in 2019, causing the Covid-19 disease. The integration of computational strategies is of great importance in the identification and development of promising new compounds. Atazanavir and Darunavir, were designed to combat resistance to mutant drugs mainly by increasing the number of polar interactions with the main atoms in the HIV protease chain. This study aims to assess the molecular interaction of the drugs Atazanavir and Darunavir with the main SARS-CoV-2 protease through docking and molecular dynamics studies. This is a descriptive, experimental study with a qualitative and quantitative approach on the subject. For that, using the programs BIOVIA Discovery Studio, PyMol, AutoDock Tools 1.5.6, AutoDock Vina, the modeling and simulation of the anchoring of the drug at the action site were carried out. Lower scores were demonstrated,

with -7.0 (Darunavir) the closest to the UAW 247 Inhibitor. It is possible to notice that the drugs showed similar residual bonds, also, in relation to the protease structure, the closest tested molecule was Atazanavir. Taking into account the stability of the RMSD values, it is valid to infer that in relation to the UAW 247 inhibitor, the drug Atazanavir is the one that best resembles, unlike Darunavir, which presents greater variations. The two drugs fit into the binding site mainly due to electrostatic interactions and hydrogen bonds. Atazanavir is the most similar to molecular activity, and Darunavir is the one with the best anchoring score.

Keywords: Molecular anchorage, Protease inhibitor, Use of medicines.

Resumen

El SARS-CoV-2 es parte de una familia de virus de ARN descrita nuevamente en 2019, que causa la enfermedad de Covid-19. La integración de estrategias computacionales es de gran importancia en la identificación y desarrollo de nuevos compuestos prometedores. Atazanavir y Darunavir, fueron diseñados para combatir la resistencia a las drogas mutantes principalmente aumentar el número de interacciones polares con átomos principales en cadena de la proteasa del VIH. Este estudio tiene como objetivo evaluar la interacción molecular de los medicamentos Atazanavir y Darunavir con la proteasa principal del SARS-CoV-2 através de estudios de acoplamiento y dinámica molecular. Este es un estudio descriptivo, experimental con un enfoque cuali-cuantitativo sobre el tema. Para ello, utilizando los programas BIOVIA Discovery Studio, PyMol, AutoDock Tools 1.5.6, AutoDock Vina, se realizó el modelado y simulación del anclaje del fármaco en sitio de acción. Se demostraron puntuaciones más bajas, siendo -7.0 (Darunavir) más cercano al inhibidor UAW 247. Es posible notar que los fármacos mostraron conexiones residuales similares, también, en relación con estructura de la proteasa, la molécula probada más cercana fue Atazanavir. Teniendo en cuenta la estabilidad de los valores de RMSD, es válido inferir que, en relación con el inhibidor de UAW 247, el medicamento Atazanavir es el que mejor se asemeja, el Darunavir, presenta mayores variaciones. Las dos drogas encajan en el sitio de unión principalmente debido a interacciones electrostáticas y enlaces de hidrógeno. Atazanavir es más similar a la actividad molecular, y Darunavir es el que tiene mejor puntuación de anclaje.

Palabras clave: Anclaje molecular, Inhibidor de protease, Uso de medicinas.

1. Introduction

In December 2019, in Wuhan, China, an emergency situation began due to outbreaks of new coronavirus (SARS-CoV-2), spreading rapidly throughout the world. This fact caused a pandemic, challenging the scientific community to develop better strategies for the treatment of this virus (Lai et al., 2020).

The coronavirus is part of an RNA virus family first described in 1965 and, in late 2019, causing the disease called Covid-19. The virus causes a clinical condition similar to a simple flu syndrome, or even asymptomatic, evidenced in some cases of younger patients or who used antipyretic medication. However, offering a risk of developing the so-called Severe Acute Respiratory Syndrome (SARS), which can lead the patient to death where the infection progresses to an advanced form of the disease (Lima, 2020).

Currently, nine clinically approved inhibitors have been developed against HIV-1 protease. These drugs are saquinavir, ritonavir, indinavir, amprenavir (also licensed as fosamprenavir), nelfinavir, lopinavir, atazanavir, tipranavir and darunavir. Second-generation inhibitors, such as atazanavir and darunavir, were designed to combat drug-resistant mutants mainly by increasing the number of polar interactions with the main atoms in the protease chain (Menéndez-Arias & Tözsér, 2008).

Cellular and biochemical processes are largely controlled by interactions between different classes of proteins. Many diseases, such as cancer, can be attributed to defective protein-protein interactions, so this type of intermolecular event is a highly attractive target in drug discovery (Ferreira et al., 2015). According to Baildya et al. (2020), the mais interactions between drug and amino acids residues are electrostatic and Van der Waals.

Molecular Docking (or molecular anchoring) method is often used in the development of structure-based drugs due to its ability to predict, with a substantial degree of precision, the anchoring and conformation of molecule ligands within the binding site at the appropriate target. The conformational search algorithm explores the energy scenario of each molecule and high-score compounds are selected as potential ligands (Ferreira et al., 2015; Uciechowska-Kaczmarzyk et al., 2019; Baildya et al., 2020).

An attractive drug target among coronaviruses is the main protease, due to its essential role in processing the polyproteins that are translated from the viral RNA (Boopathi et al., 2020). According to Ferreira et al. (2015), the integration of computational and experimental strategies has been of great value in the identification and development of promising new compounds. Today, there is a wide variety of plug-in algorithms available, but understanding

of the advantages and limitations of each method is fundamental in the development of effective strategies and in the generation of relevant results. In the present work, the molecular interaction of Atazanavir and Darunavir with the main protease of Covid-19 (PDB ID: 6XBH) were evaluated by molecular docking study.

2. Methodology

2.1. Methodological procedures

This is a descriptive, observational, experimental study with a qualitative and quantitative approach on the topic (Pereira et al., 2018). The main protease molecule of Covid-19 was obtained from the Protein Data Bank (PDB) database, whose identification code is 6XBH. Tables were made using Microsoft Excel® 2010 software.

2.2. Anchoring of Atazanavir and Darunavir in the main protease of SARS-CoV-2

Were used the methodologies described by Wang et al. (2015), with modifications made by Sousa et al. (2020). Structures of the 3D conformers of Darunavir (CID: 213039) and Atazanavir (CID: 148192) were obtained through PubChem. The protease structure obtained from the PDB was cleaned, by removing all water molecules, hetero groups and the previous ligand (UAW 247) using BIOVIA Discovery Studio. AutoDock Tools 1.5.6 software was used to prepare the ligands, followed by PyMol, used to create and visualize the protease-ligand complex. According to Trot & Olson (2010), anchoring simulations were performed by selecting the connection location previously listed with the AutoDock Vina package.

2.3. Analysis of molecular dynamics simulations

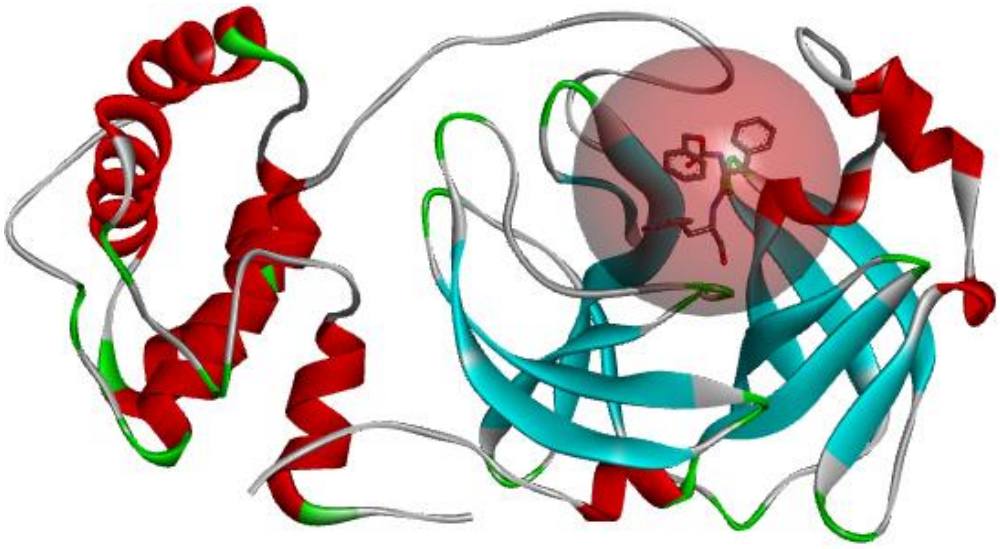
According to Baildya et al. (2020), gromacs software with the tools trjconv and gmx energy were used for the protease-ligand complex, to replace the protease and drug molecules inside the cubic box, and to calculate the interaction energy values respectively. Plots of the deviation of the Root-mean-square deviation (RMSD) were made using the QtGrace software.

3. Results and discussion

3.1. Molecular anchorage studies

After being isolated, the main protease molecule of SARS-CoV-2 was submitted to BIOVIA Discovery Studio, to select the correct the possible binding site for the molecular docking study, represented in Figure 1 in a reddish spherical form, with the UAW 247 inhibitor inside.

Figure 1. Location for performing molecular docking.



Source: BIOVIA Discovery Studio (2020).

The ligand was subsequently removed after elucidating the coordinates of the location of the active site (inside the red ball in Figure1).

In the conformational search stage, structural parameters of the ligands, such as torsion (dihedral), degrees of freedom of translation and rotation, are incrementally modified. Conformational search algorithms perform this task by applying systematic and stochastic search methods. Systematic search methods promote small variations in structural parameters, gradually changing the conformation of the ligands. The algorithm investigates the energy scenario of the space conformational system and, after numerous cycles of research and evaluation, converges to the minimum energy solution corresponding to the most likely connection mode (Ferreira et al., 2015; Bursulaya et al., 2003). The anchoring socres of the UAW 247 inhibitor, Atazanavir and Darunavir its show in the Table 1.

Table 1. Anchoring scores of the tested molecules.

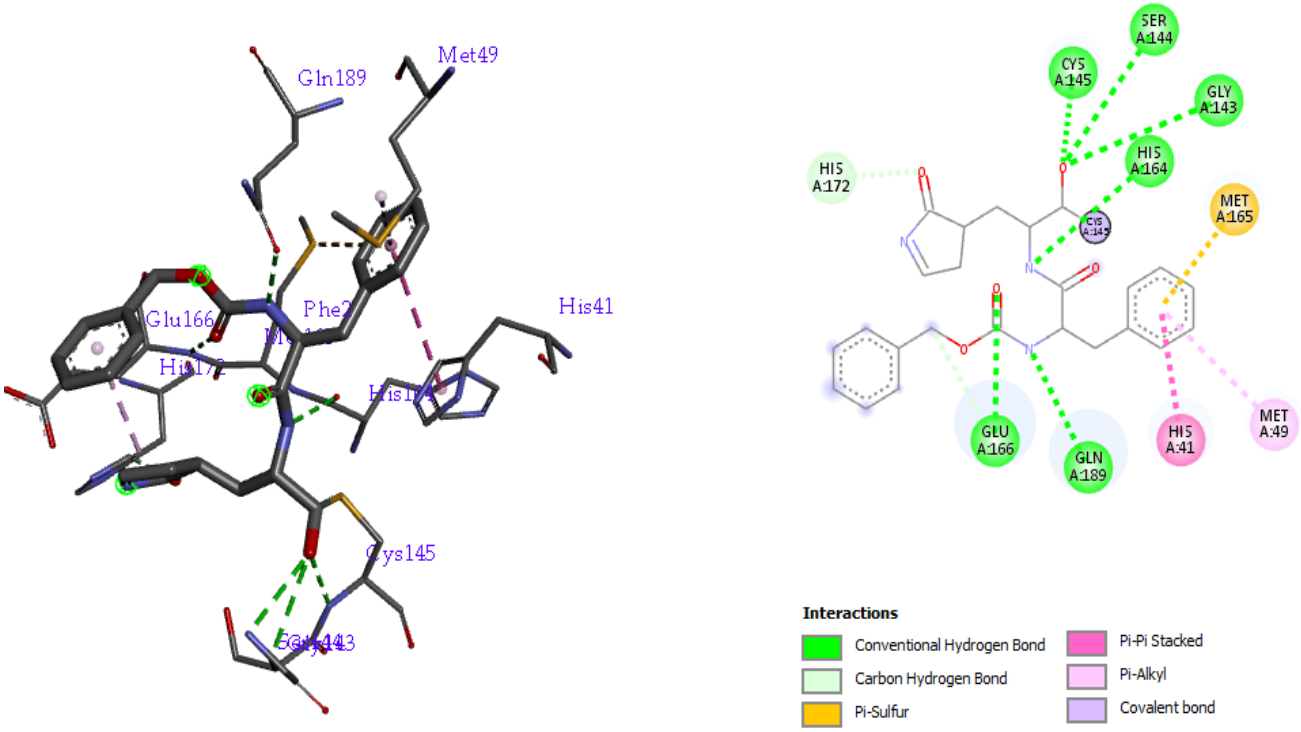
Structures anchored in the protease	Anchorage score (kcal / mol)
UAW 247 inhibitor	-7,21
Atazanavir	-6.0
Darunavir	-7.0

Source**:** Prepared by the authors (2020).

It is possible to observe slightly lower scores were shown in Table 1, Darunavir (-7.0 kcal / mol), which approximates the UAW 247 inhibitor, a fact similar to the results found by Mittal et al. (2020). The coupling power in the protease is assessed by measuring the coupling score (Baildya et al., 2020; Uciechowska-Kaczmarzyk et al., 2019; Ferreira et al., 2015).

In Figure 2, it is possible to observe the amino acid residues between the UAW 247 inhibitor and the molecular structure of the SARS-CoV-2 protease.

Figure 2. UAW 247 inhibitor protease-ligand interactions.

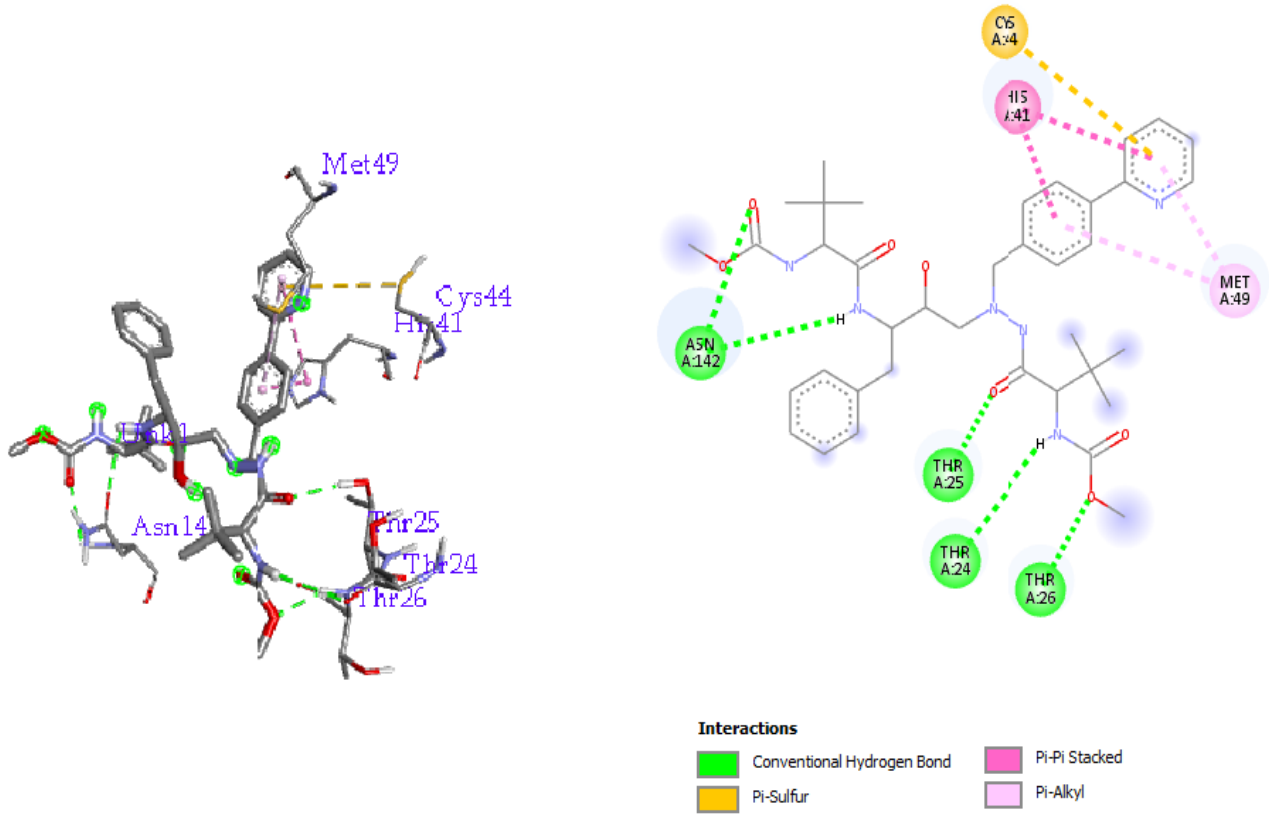


Source: BIOVIA Discovery Studio (2020).

From the structure represented in 3D, as well as the 2D diagram, both created with BIOVIA Discovery Studio, it is possible to notice that the neighboring groups are present in the structure of the main SARS-CoV-2 protease in the complex with UAW 247 inhibitor are SER144, CYS145, GLN189, GLY143, GLU166, MET49, MET165 HIS164, HIS172 and HIS41.

In Figure 3, it is possible to observe the amino acid residues between the Atazanavir and the molecular structure of the SARS-CoV-2 protease.

Figure 3. Atazanavir protease-ligand interactions.

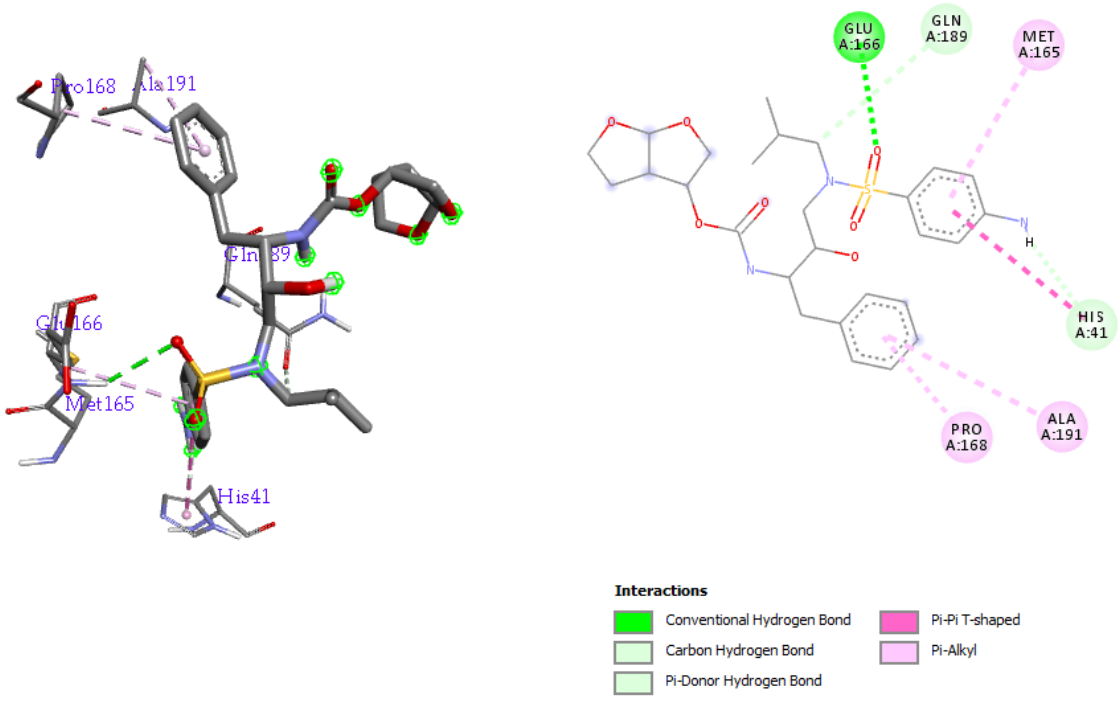


Source: BIOVIA Discovery Studio (2020).

From the structure represented in 3D, as well as the 2D diagram, both created with BIOVIA Discovery Studio, it is possible to notice that the neighboring groups are present in the structure of the main SARS-CoV-2 protease in the complex Atazanavir are HIS41, MET49, CYS44, THR24, THR25, THR26 and ASN142. It is possible to observe that, there are similar interaction residues in relation to the UAW 247 inhibitor, they are HIS41 and MET49.

In Figure 4, it is possible to observe the amino acid residues between the Darunavir and the molecular structure of the SARS-CoV-2 protease.

Figure 4. Darunavir protease-ligand interactions.



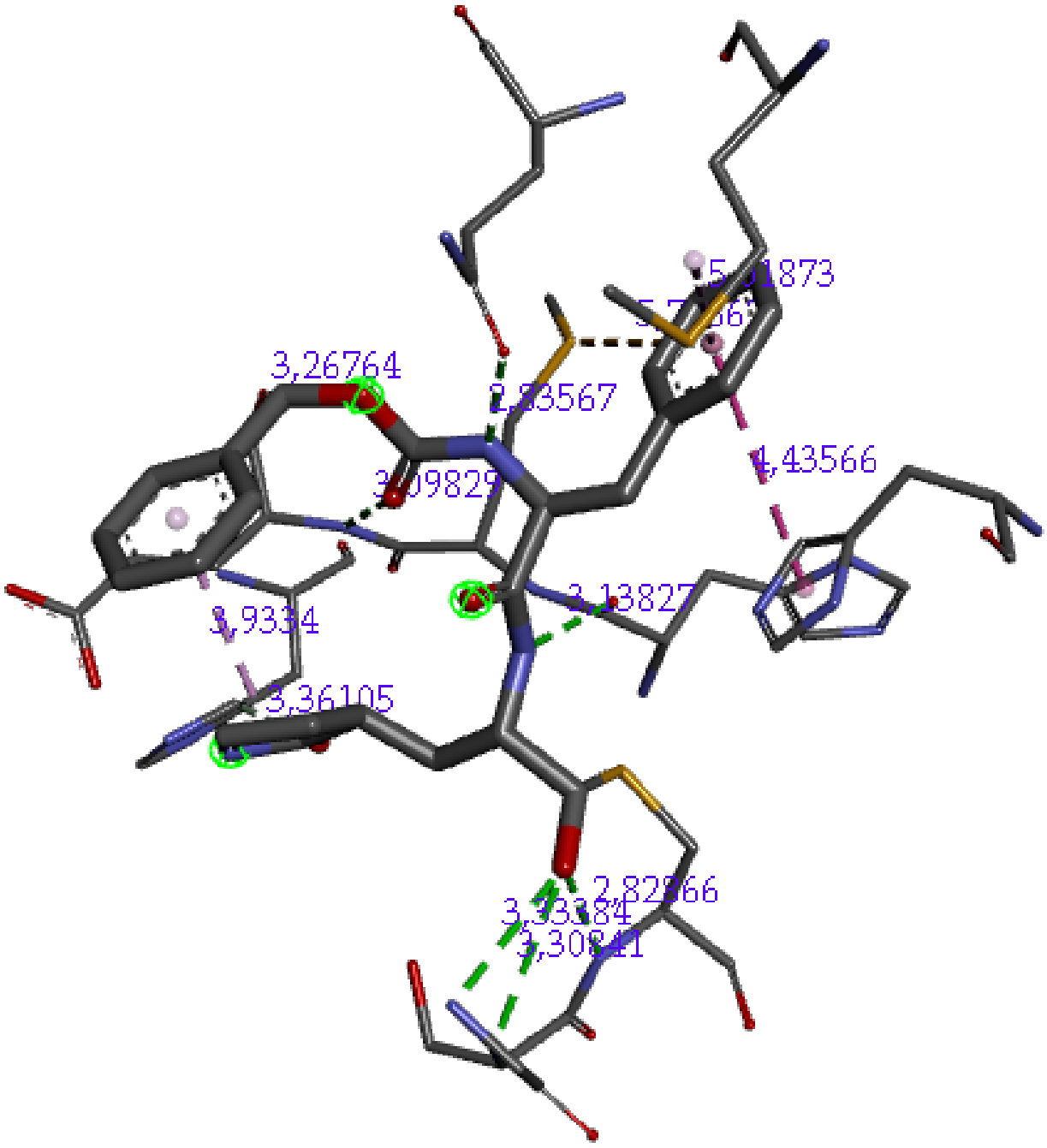
Source: BIOVIA Discovery Studio (2020).

The anchored Darunavir was surrounded by the GLU166, GLN189, MET165, HIS41, ALA191 and PRO168 residue. It is possible to notice that the Darunavir showed more connections with common amino acid residues, justifyng an anchoring score similar to that achieved with the UAW 247 inhibitor, which shares the same residues GLU166, GLN189, MET165 and HIS41.

3.2. Molecular dynamics simulation studies

Using the BIOVIA Discovery Studio software, it was possible to visualize the distance between the connections presented by the UAW 247 inhibitor after anchoring in the SARS-CoV-2 protease, represented in Figure 5.

Figure 5. Angstrom distance (Å) of the UAW 247 inhibitor.

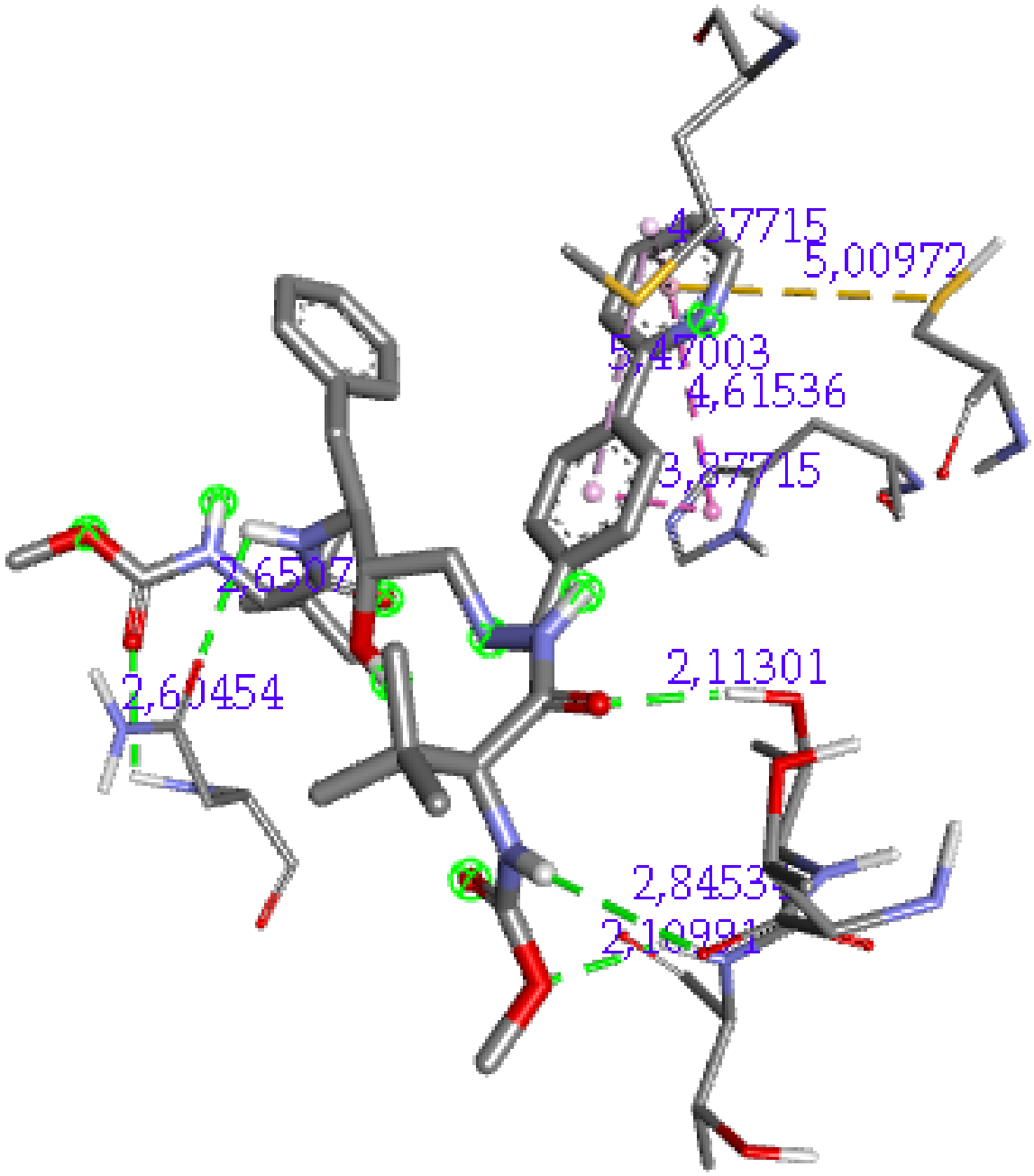


Source: BIOVIA Discovery Studio (2020).

After analyzing the distance variations in the angstrom (Å) of the ligands tested in relation to the amino acid residues present in the protease molecule, represented in Figure 5, it was possible to calculate the average distance, with the value of 3,69Å for the structure of the UAW inhibitor 247.

Using the BIOVIA Discovery Studio software, it was possible to visualize the distance between the connections presented by the Atazanavir inhibitor after anchoring in the SARS-CoV-2 protease, represented in Figure 6.

Figure 6. Angstrom distance (Å) of the Atazanavir.



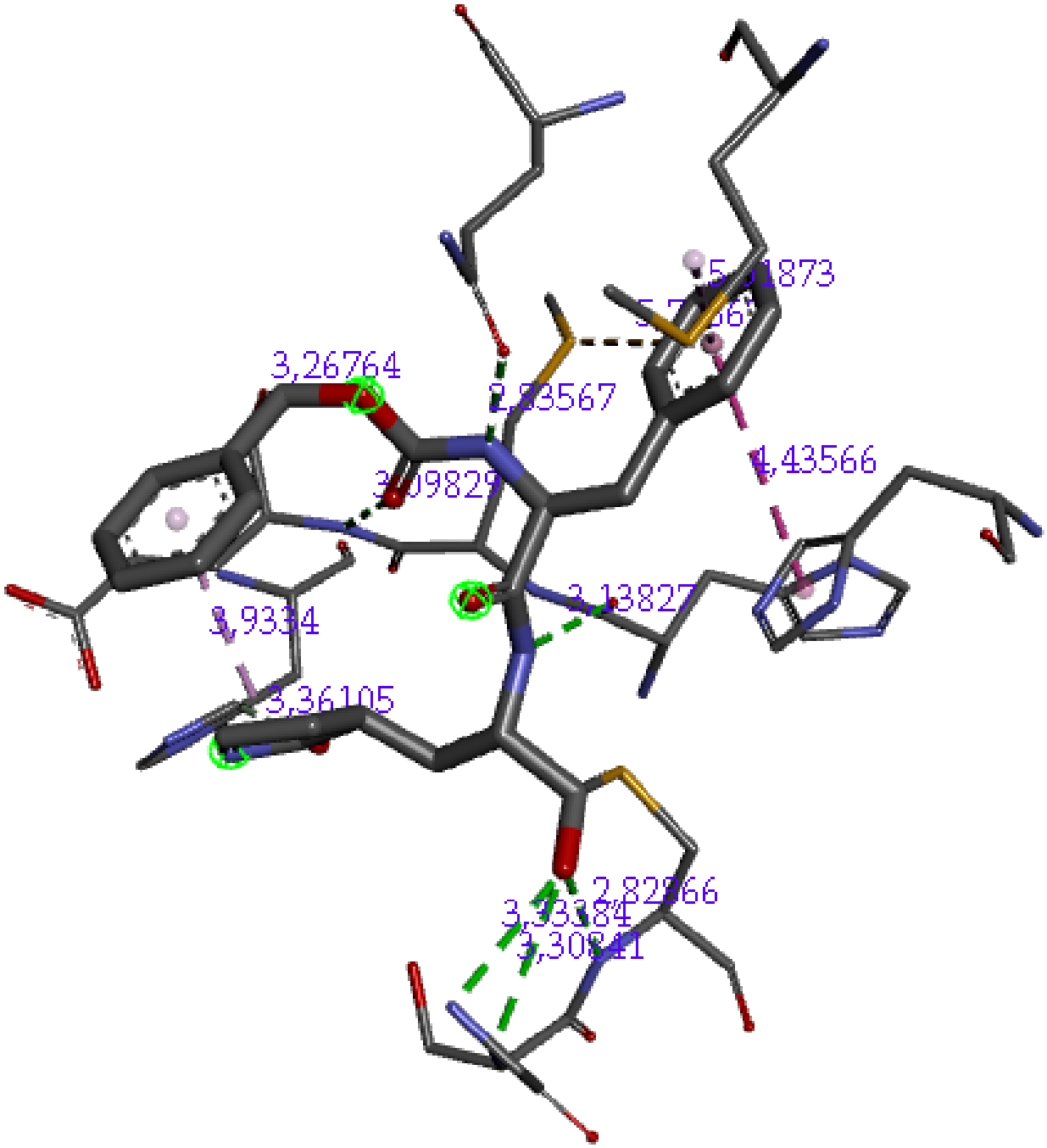
Source: BIOVIA Discovery Studio (2020).

After analyzing the distance variations in the angstrom (Å) of the ligands tested in relation to the amino acid residues present in the protease molecule, represented in Figure 6, it

was possible to calculate the average distance, with the value of 3,6Å for the structure of the Atazanavir.

Using the BIOVIA Discovery Studio software, it was possible to visualize the distance between the connections presented by the Darunavir inhibitor after anchoring in the SARS-CoV-2 protease, represented in Figure 7.

Figure 7. Angstrom distance (Å) of the Darunavir.



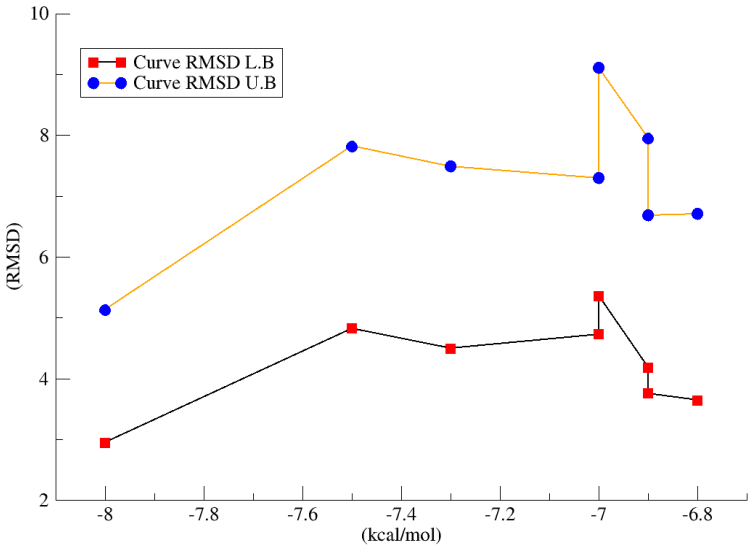
Source: BIOVA Discovery Studio (2020).

After analyzing the distance variations in the angstrom (Å) of the ligands tested in relation to the amino acid residues present in the protease molecule, represented in Figure 7, it was possible to calculate the average distance, with the value of 4,16Å for the structure of the Darunavir. Therefore, it is clear that in relation to the protease structure, despite the proximity between the results, the test molecule closest to the protease structure was Atazanavir.

Root-mean-square deviation (RMSD) values represent stability and molecular variations This parameters are calculated in order to provide two metric variants, upper bound (UP) and lower bound (LB), for each fitting method, and only the best score per complex was saved. If the ligand has local topological symmetry in a single connection, whose torsion angle can be altered by a rotation less than 360º without alterations in the ligament conformation, the RMSD of alternative orientations is calculated, keeping the lowest for comparison purposes. The coordinates of the ligand structure, used for RMSD calculations, are obtained by overlapping the coordinates of crystallographic proteins with the coordinates of the protease-ligand complex used for the fitting (Trot & Olson, 2010; Bursulaya et al., 2003).

After plotting the results graphically using the QtGrace software, the RMSD data is represented in Figure 8 for the UAW 247 inhibitor in relation to the protease molecule of SARS-CoV-2.

Figure 8. Graph of the mean square root deviation (RMSD) of the receptor (protease) and ligand UAW 247.

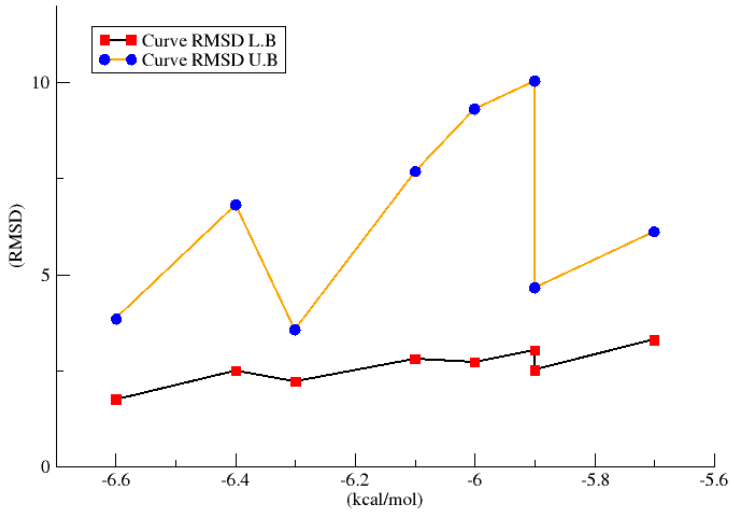


Source: Prepared by the authors (2020).

After observing the data exposed in Figure 8, referring to the UAW 247 inhibitor, parameters were established comparatively in relation to it, based on this ligand that was previously allocated. It is possible to observe that the plotted graph shows few variations in relation to the uniform difference in values between the L.B and U.B.

After plotting the results graphically using the QtGrace software, the RMSD data is represented in Figure 9 for the Atazanavir in relation to the protease molecule of SARS-CoV-2.

Figure 9. Graph of the mean square root deviation (RMSD) of the receptor (protease) and ligand Atazanavir.

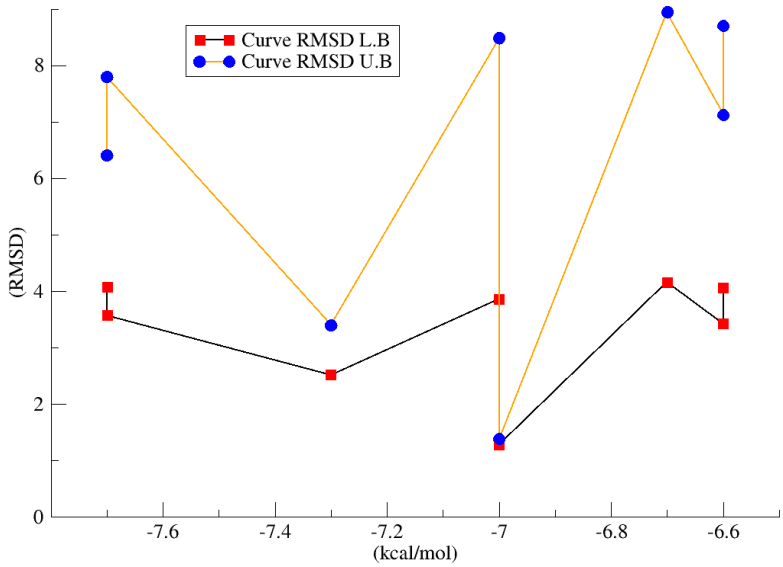


Source: Prepared by the authors (2020).

After analyzing the RMSD values, it was observed that there are variations as new structural conformations appear, with a considerable structural change within a short period of energy variation. Therefore, considering the stability of the values, it is valid to infer that, in relation to the UAW 247 inhibitor, Atazanavir is the one that best resembles.

After plotting the results graphically using the QtGrace software, the RMSD data is represented in Figure 10 for the Darunavir in relation to the protease molecule of SARS-CoV-2.

Figure 10. Graph of the mean square root deviation (RMSD) of the receptor (protease) and ligand Darunavir).



Source: Prepared by the authors (2020).

After analyzing the RMSD values, it was also observed that there are variations as new structural conformations emerge, with a considerable structural change within a short period of energy variation. In contrast to Atazanvir, and compared to the UAW 247 inhibitor, Darunavir showed noticeably greater variations in relation to RMSD values, suggesting greater intabilities in relation to the SARS-CoV-2 protease molecule.

4. Conclusion and Suggestions

This research involves the study of the interaction between Atazanavir and Darunavir, HIV protease inhibitors, and the referred protease of SARS-CoV-2, in comparision to alleged inhibitor of this protein. The molecular coupling study reveals that both Atazanavir and Darunavir fit into the binding site due to mainly electrostatic and hydrogen interactions, with an anchoring score similar to that of the inhibitor before allocated.

It is also worth clarifying that Atazanavir is the one that most closely resembles the molecular activity of the UAW 247 inhibitor, and Darunavir is the one with the best anchoring score. However, it is possible to establish that it is necessary to carry out in vitro and in vivo, studies in order to possibly corroborate these findings.

After the exposed researches, it is possible to define the tests *in vitro* and *in vivo*, they are essential for these findings, given that they are presented as a way to confirm the results presented.

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