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Article

Investigation of dual anti-HIV/HSV activity of oxoquinoline-acylhydrazone derivatives by molecular docking

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Some oxoquinoline-acylhydrazone derivatives showed activity against Human Immunodeficiency Virus type 1 (HIV-1). These compounds must also be active against Herpes Simplex Virus type 1 (HSV-1) by an inhibition mechanism where they interact with the HSV-DNA-polymerase/DNA-duplex complex. There are several treatment options for HSV-1 but there is no cure for the disease, which may represent a life risk for individuals co-infected with HIV. In this work molecular docking studies were carried out in an attempt to understand the dual activity of these oxoquinoline-acyhydrazone derivatives. The compounds were docked in two possible situations: (i) in the polymerase domain of HIV-1 Reverse Transcriptase (RT) enzyme in order to verify whether the inhibition occurs similarly to the proposed mechanism for HSV-1 inhibition, where the ligand would form a complex with the enzyme and the DNA; (ii) in the allosteric site of RT in order to verify if the inhibition occur in a similar way to non-nucleoside RT inhibitors (NNRTI). The studied compounds showed higher binding affinity to the allosteric site of RT and the results indicate that the inhibition should occur in a mechanism similar to that of NNRTI, which produces an allosteric inhibition that induces structural changes in the enzymatic active site.

Keywords: Molecular docking. HIV-1. HSV-1. Oxoquinoline-acylhydrazone derivatives. Dual inhibitors.

INTRODUCTION

Herpes Simplex type 1 (HSV-1) is a double-stranded DNA virus that spreads through nerve pathways and causes infections mainly in the skin and orofacial mucosa. Symptoms are small reddish lesions on the mucosa that can cause extremely painful yellowish ulcers. There are several treatment options to HSV-1 but most of them are palliative measures since there is no cure for the disease (Fatahzadeh, Schwartz, 2017).

Some antiviral drugs are able to shorten the duration of the illness and prevent flare-ups. These drugs work by decreasing the virus replication rate, helping the immune system to interfere. Acyclovir (ACV) is the most widely used antiviral drug for the treatment of HSV-1. However the virus generates some drug resistance, especially among immunocompromised patients (Gopinath *et al*., 2023).

The resistance to ACV is due to mutations in thymidine kinase or DNA polymerase enzymes that are involved in the mechanism of action of this drug. In this context the searching for new drugs is an important strategy mainly because of the life risk for co-infected Human Immunodeficiency Virus (HIV) individuals. In addition, in immunocompromised patients, herpetic infections can cause severe complications (Gangemi *et al*., 2021).

HIV was recognized in 1983 as the causative agent of the Acquired Immunodeficiency Syndrome (AIDS) and continues to be a worldwide health care issue. HIV has two known variants: HIV-1 which causes HIV infections worldwide, and HIV-2 mostly confined to West Africa, which causes an attenuated infection compared to HIV-1 (Visseaux *et al*., 2019).

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The Reverse Transcriptase (RT) enzyme is the main target of HIV-1 antiretroviral drugs because it is a multifunctional enzyme in the life cycle of the virus. Similar to other polymerases the RT polymerase domain has a hand-like conformation with fingers (residues 1-85 and 118-155), palm (residues 86–117 and 156–236) and thumb (residues 237 –318), in addition to the connection (residues 319–426) with subdomains. Structures and biophysical studies reveal that RT is highly flexible and adapts with spatial rearrangements to perform its functions (Singh, Das, 2022).

Nucleoside Reverse Transcriptase inhibitors act as DNA chain finishers through a competitive mechanism, binding to the catalytic site and blocking viral DNA synthesis. On the other hand non-nucleoside Reverse Transcriptase inhibitors (NNRTI) act by interacting at a different site, producing an allosteric inhibition that induces structural changes in the enzymatic active site (Cunico, Gomes, Vellasco, 2008).

Nevirapine (**NVP**) is as an example of NNRTI which has a direct impact on the RT-DNA complex. With its binding the primer claw is displaced and accordingly the terminal primer moves outward. Because of this the thumb is locked in a hyperextended position, resulting in loss of interactions between the DNA and the polymerase domain. The deoxyribonucleotide phosphate (dNTP) binding site is distorted and the RNase H active site is repositioned relative to the thumb active site of the polymerase (Das *et al*., 2012).

Over the years new drugs have been developed to aid in the allosteric inhibition of this enzyme, and currently there are six non-nucleoside analogue drugs approved for the treatment of HIV-1 infections: nevirapine, delavirdine, efavirenz, etravirine, rilpivirine and doravirine. Some of these structures are shown in Figure 1. The discovery of etravirine and rilpivirine brought a new concept of using conformational flexibility in drug design to overcome the impact of drug resistance mutations. Torsional flexibility can help in drug reorientation and reposition to maintain efficacy even in face of active site mutations. Thus, ligands created from the junction of flexible fragments with structures with biological activity can bind to the active site, limiting the natural functions of RT and consequently, inhibiting the enzyme, decreasing resistance profiles (Singh, Das, 2022).

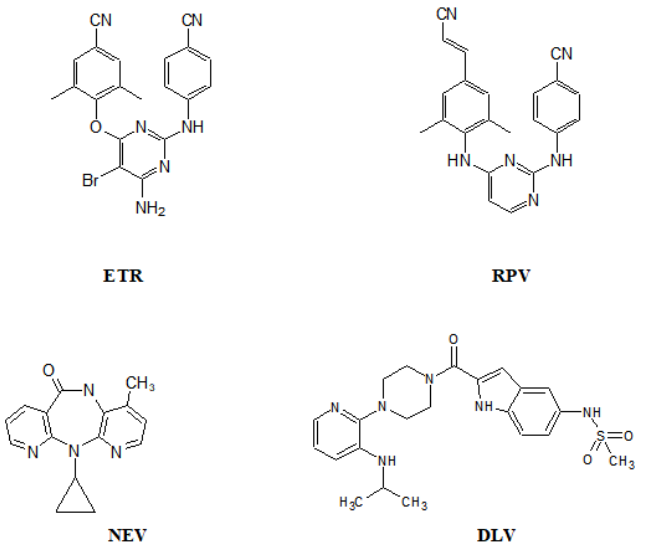

FIGURE 1- Molecular structures of some NNRTI in current use for HIV-1 treatment: etravirine (**ETR**), rilpivirine (**RPV**), nevirapine (**NVP**) and delavirdine (**DLV**).

Oxoquinoline-acylhydrazone derivatives (**1-3**, Figure 2) are active against HIV-1 and also must be active against HSV-1 (Yoneda *et al*., 2014). One of the limitations of the drugs available for the treatment of Herpes is that they are only active against some of the eight Human Herpes viruses. Pharmacia Corp. synthesized, tested and studied several 4-dihydro-oxoquinoline compounds, such as PNU-183792 (**4**, Figure 2) (Thomsen *et al*., 2003). The antiviral activity of these non-nucleoside inhibitors correlates with inhibition of viral DNA polymerase. These compounds showed some positive aspects: they are selective, that is, they inhibit viral DNA polymerases without inhibiting human DNA polymerases, they are active against viruses resistant to ACV and they prevent the replication of a wide spectrum of Herpes viruses. The latter can be explained by the fact that they interact with a region within domain III of the viral DNA polymerase, a domain that is extremely conserved in many Herpes viruses (Thomsen *et al*., 2003).

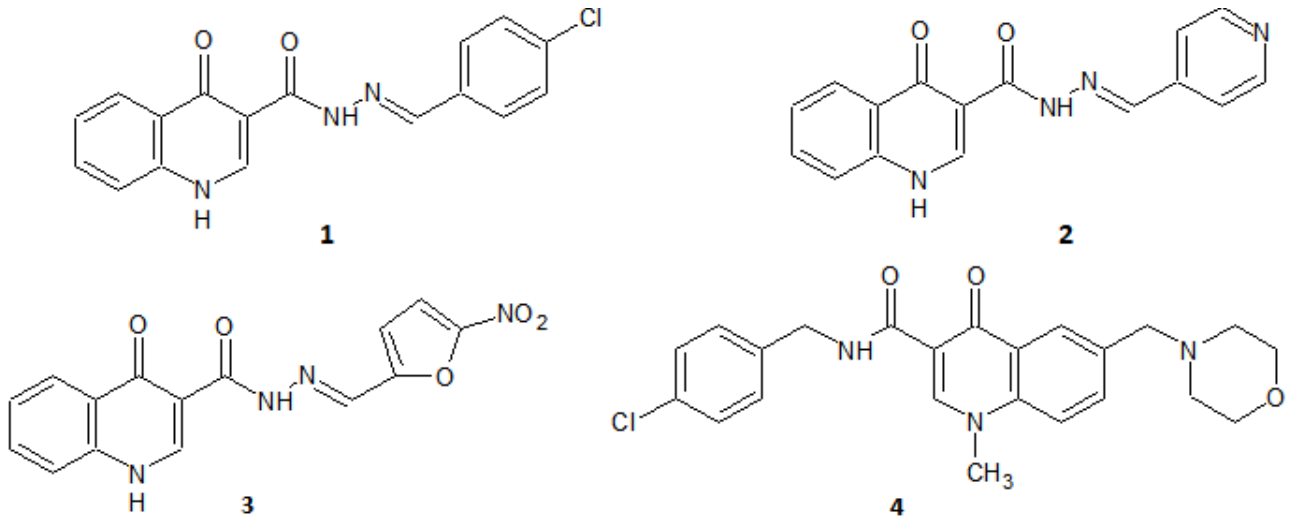

FIGURE 2 - Molecular structures of oxoquinoline-acylhydrazone derivatives active against HIV-1 (**1**-**3**) and PNU-183792 (**4**), active against HSV-1, and used as a model in the literature, for understanding the activity of this class of compounds in the HSV-1 DNA polymerase.

Compounds **1**-**3** have structural similarities with compound **4**. Molecular modelling studies indicates that they should interact with HSV-DNA-polymerase/DNA-duplex complex in an uncompetitive inhibition mechanism, according to the model proposed by Liu and co-workers (2006) for **4** (Yoneda *et al.*, 2014). In this model the ligand displaces a template base from the active site by replacing a nucleotide. The oxoquinolinic ring of PNU-183792 is "stacked" with the aromatic rings of the nitrogenous bases of the primer/template and it also interacts with amino acid residues of the polymerase (Liu *et al.*, 2006).

In this work molecular docking studies of compounds **1**, **2** and **3** with HIV-1 RT were carried out in order to evaluate the mechanism of action which could explain the anti HIV-1 activity observed by Yoneda and co-workers (2014). Two possible mechanisms were evaluated: (i) inhibition of RT in the polymerase domain of the enzyme, according to the model proposed by literature for the inhibition of HSV-1 DNA polymerase (Liu *et al.*, 2006); (ii) inhibition at the RT allosteric site like the NNRTIs, as nevirapine (**NVP**) and delavirdine (**DLV**), for example.

MATERIAL AND METHODS

The structures of the HIV-1 RT (PDB ID: 1KLM), HIV-1 RT complexed with DNA (PDB ID: 3V6D), and of the HIV-1 RT complexed with DNA and nevirapine (PDB ID: 3V81) (Das *et al.*, 2012) were obtained from the Protein Data Bank (Berman *et al.,* 2000).

The docking simulations were carried out using CLC Drug Discovery program (CLC Drug Discovery Workbench, 2014). The ligands were designed in CLC Drug Discovery by using the ligand optimizer tool. They were flexibly docked in all calculations performed. The best score of each ligand was evaluated.

In the first stage of this work the preparation of the binding site was performed on the 3V6D enzyme by selecting Tyr271, Ile270, Trp266, Asn265 and Lys259 residues from the RT polymerase domain, which shows analogy with region III of the HSV-1 DNA polymerase. The residue Tyr181 was also selected, since it is one of the main amino acids contributing to the high binding affinity of new families of non-nucleoside RT inhibitors. The region III corresponds to the amino acid sequence of the C-terminal end (residues 256-271) of the RT polymerase domain (Lindborg, 1992). The binding site obtained by this selection, with a radius of 13 Å, includes part of the DNA.

In the second stage the binding site was centered on the ligand (**NVP**) present in the enzyme 3V81, with the maximum radius of 25 Å. In this way, it was possible to evaluate, among several possibilities, which

would be the preferred interactions between ligand and receptor since the program analyzes the ligand in all cavities and shows the positions with the most stable interactions. The docking score used in the CLC Drug Discovery Workbench is the $PLANTS_{PLP}$ (Korb, Stützle, Exner, 2009).

RESULTS AND DISCUSSION

Initially, nevirapine and delavirdine were redocked with 3V81 and 1KLM enzymes respectively, centralizing the binding site in the allosteric site of the ligand. The results reproduced all the interactions observed experimentally, with a rootmean-square deviation (RMSD) of 1.07 and 1.02 Å respectively, and support the hypothesis that the experimental binding mode could be reproduced by this approach.

The first stage of this work aims to evaluate if the mechanism proposed by Liu and co-workers (2006) for the inhibition of HSV-1 DNA polymerase could be applied to the comprehension of the inhibition of HIV-1 RT by the oxoquinoline-acylhydrazone derivatives, verifying the interactions and scores of the ligands when binding simultaneously to the enzyme and to the DNA. The binding site (radius of 13 Å) included only one cavity in 3V6D, located close to the residues that showed analogy with region III of the HSV-1 DNA polymerase (Lindborg, 1992) and close to the DNA. The calculations were performed for five ligands (**1**, **2**, **3**, **NVP** and **DLV**) and the results are found in Table I.

TABLE I - Interactions and scores obtained for the docking of compounds **1**, **2**, **3**, **NVP** and **DLV** with 3V6D in the first stage of the work. Residues in orange show analogy with region III of HSV DNA polymerase

	NVP	1	2	3	DLV
Ile94		X		X	X
Pro95					X
His96	X	X	X	X	X
Pro97					X
Met230					X
Tyr232					X
Lys259					
Val261					
Gly262	X	X	X	X	
Lys263					
Leu264					
Asn265	X	X	X	X	X
Trp266	X	X	X	X	X
Ala267	X				
Ser268	X	X	X	X	X
Gln269	X	X	X	X	X
Ile270					

TABLE I - Interactions and scores obtained for the docking of compounds **1**, **2**, **3**, **NVP** and **DLV** with 3V6D in the first stage of the work. Residues in orange show analogy with region III of HSV DNA polymerase

	NVP	1	2	3	DLV
Tyr271					
Tyr339	X	X	X	X	X
Lys350	X	X	X	X	X
Thr351		X	X	X	
Gly352	X	X	X	X	X
Lys353	X	X	X	X	X
Tyr354					X
Lys374	X	X	X	X	X
Glu378	X	X	X	X	X
Val381		X		X	
Ile382	X	X	X	X	X
G708					X
C709		X	X	X	X
G710	X	X	X	X	X
C711	X	X	X	X	X
G819	X	X	X	X	X
C820	X	X	X	X	X
C821					X
Score	-46.88 ± 0.01	**-54.72** ±0.10	-53.63 ± 0.13	-52.19 ± 0.09	-31.39 ± 0.97

Compound **1** had the best score among all compounds studied and a total of 16 interactions with the enzyme (Figure 3). Of these interactions 13 were similar to that observed for **NVP** (Figure 4) and 13 were similar to that observed for **DLV** (highlighted in green, Table I), that is, the ligands (studied ligands and reference ligands) interact with the receptor in a similar way. Interactions with DNA were the same for compounds **1**, **2** and **3**, and the interactions with amino acids did not show great difference, ranging from 14 to 17 residues. The three compounds performed 14 interactions in common.

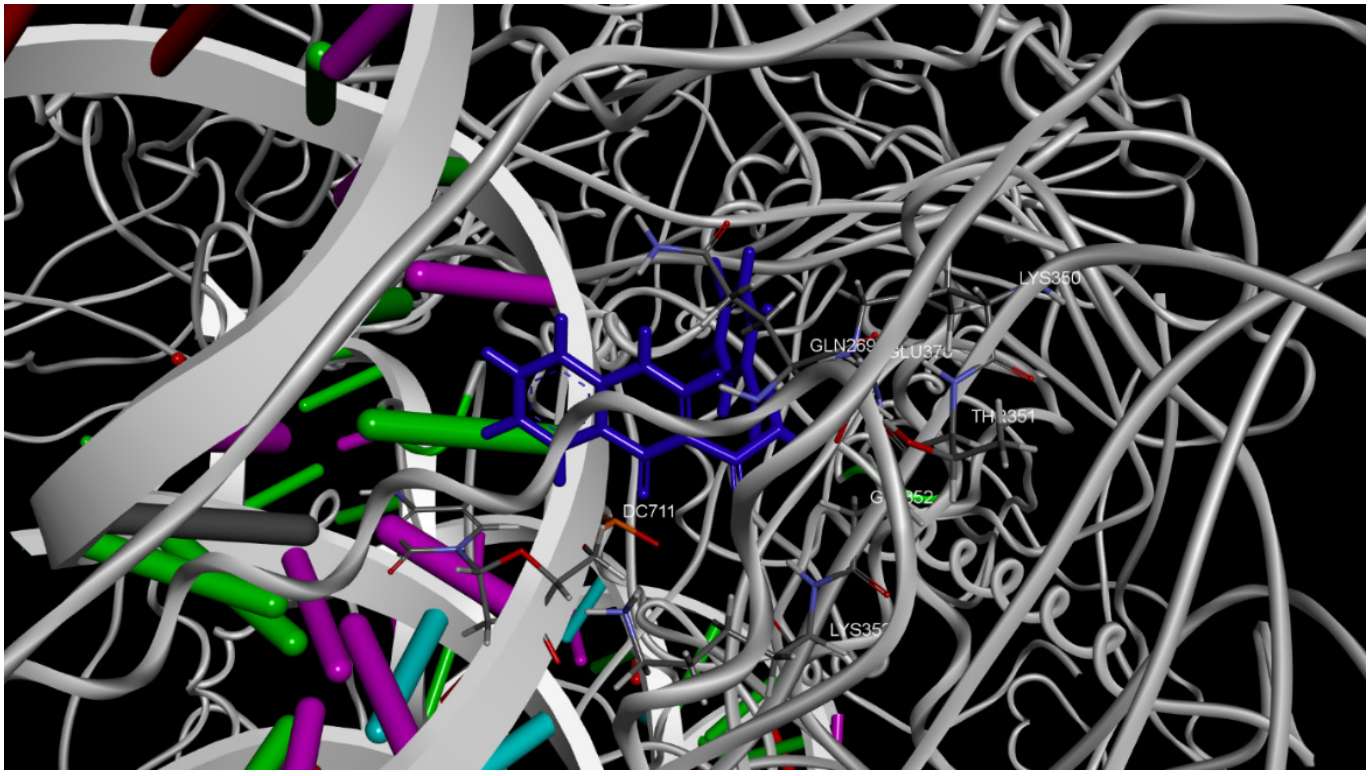

FIGURE 3- Best docking result for compound **1** (in blue) in the polymerase domain of HIV-1 Reverse Transcriptase.

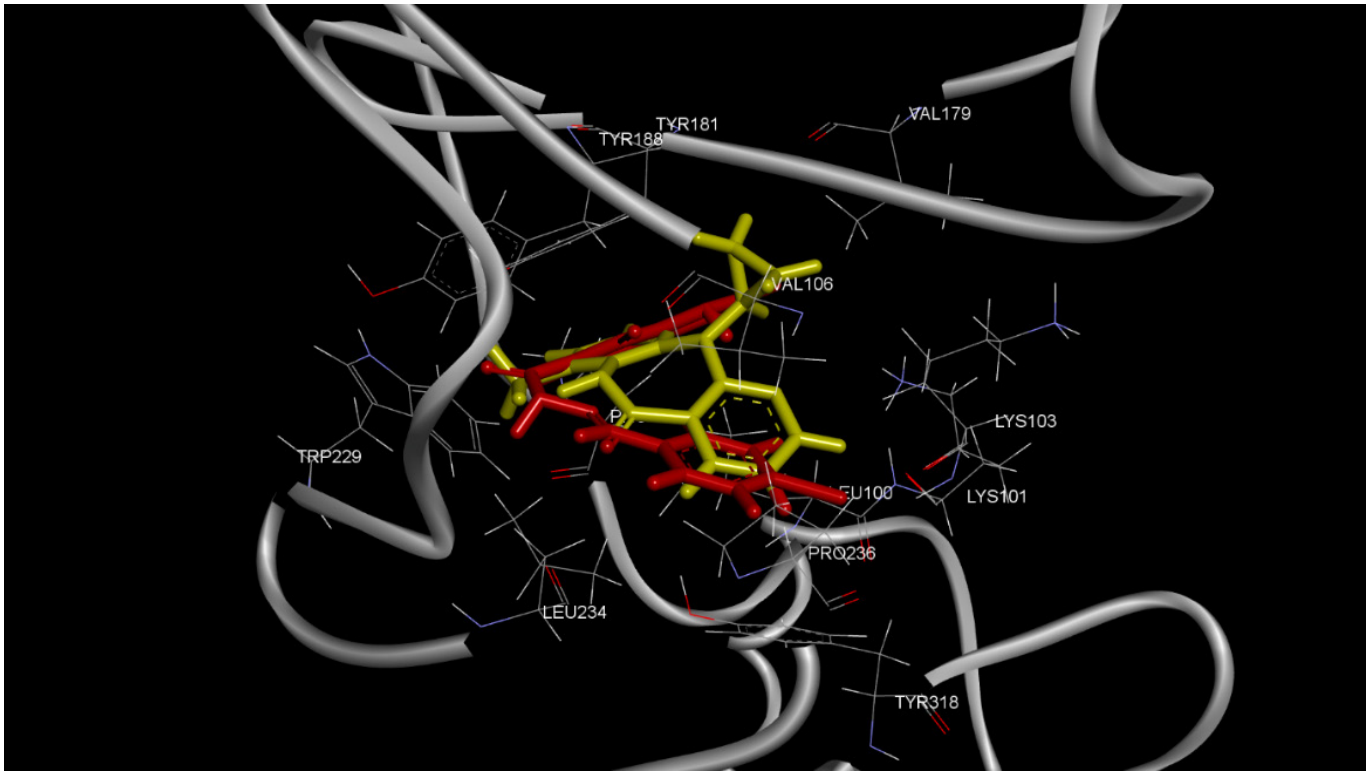

FIGURE 4- Best docking result for compound **1** (in red) superimposed with **NVP** (in yellow) in the allosteric site of HIV-1 Reverse Transcriptase.

In the second stage of the work, the docking of **1**, **2**, **3**, **NVP** and **DLV** was performed with the structure 3V81. The chosen binding site of 25 Å included a total of 8 cavities. The results are shown in Table II, and Figure 5 illustrates the best pose for compound **1** in the second studied site.

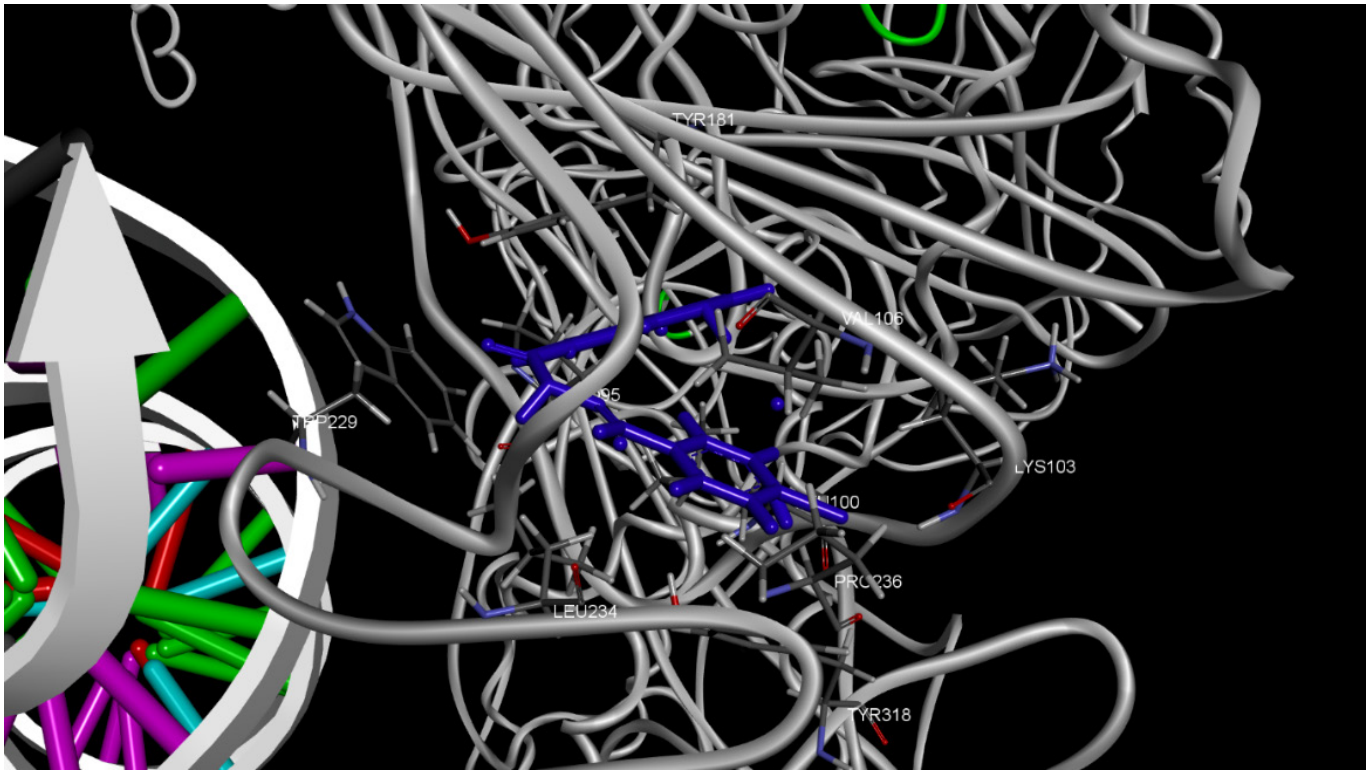

FIGURE 5- Best docking result for compound **1** (in blue) in the allosteric site of HIV-1 Reverse Transcriptase.

TABLE II - Interactions and scores obtained for the docking of compounds **1**, **2**, **3**, **NVP** and **DLV** with 3V81 in the second stage of the work

	NVP	1	2	3	DLV
Pro95	X	X	X	X	X
Leu100	X	X	X	X	X
Lys101	X	X	X	X	X
Lys102	X	X	X	X	X
Lys103	X	X	X	X	X
Lys104					X
Ser105	X				X
Val106	X	X	X	X	X
Asn136	X	X	X		
Asn137		X	X		X

TABLE II - Interactions and scores obtained for the docking of compounds **1**, **2**, **3**, **NVP** and **DLV** with 3V81 in the second stage of the work

	NVP	1	2	3	DLV
Glu138	X	X	X	X	
Val179	X	X	X	X	X
Ile180	X	X	X	X	
Tyr181	X	X	X	X	X
Tyr188	X	X	X	X	X
Val189	X				X
Gly190	X				X
Glu191	X				
Glu224					X
Pro225					X
Pro226					X
Phe227	X	X	X	X	X
Trp229	X	X	X	X	X
Leu234	X	X	X	X	X
His235	X	X	X	X	X
Pro236	X	X	X	X	X
Asp237	X	X	X	X	
Tyr318	X	X	X	X	X
Score	-68.38 ± 0.02	-65.37 ± 0.13	-67.05 ± 0.13	**-70.47 ± 0.24**	-65.58 ± 0.18

The NNRTI allosteric site is not so close to the DNA, and for this reason interactions with the nitrogenous bases were not observed in Table II. The scores for ligands **1**, **2** and **3** were close to that of the reference compounds (**NVP** and **DLV**). Therefore, the interactions with the residues from allosteric site are different from that presented for compounds **1**, **2** and **3** (Table II). **NVP** and **DLV** showed 23 interactions with the enzyme while compounds **1**, **2**, **3** showed 20, 20 and 18, respectively. Although they present a smaller number of interactions, the ones presented by them should be the crucial ones for the inhibition, since they are active against HIV-1 (Yoneda *et al.*, 2014).

The scores of - 46.88 for **NVP** and of - 31.39 for **DLV** in Table I were the highest among the compounds studied for that site, indicating a binding energy not as stable as that of the compounds **1**, **2**, and **3.** According to experimental data the inhibition of such drugs takes place at the allosteric site (PDB id 3V81 for nevirapine and 1KLM for delavirdine), therefore their lower stabilities at another site were expected. All scores in Table II show greater stability compared to those in Table I, indicating that ligands tend to bind at the second site studied. The results lead to the conclusion that the HIV-1 RT inhibition mechanism of oxoquinoline-acylhydrazone derivatives studied should be similar to that of **NVP** and **DLV**, and thus, observed anti HIV-1 activity of **1**, **2**, and **3** (Yoneda *et al.*, 2014) may be explained by their action like NNRTIs

in the allosteric site and not by a complex formation with DNA and residues in the RT polymerase domain.

It is interesting to note that mutations for Tyr318 have not been described yet in literature. The nitro group of compound **3** interacts with this residue by hydrogen bond (Figure 6). Since the allosteric site of HIV-RT is hydrophobic the interaction contributes to complex stabilization, and compound **3** may be less susceptible to viral resistance (Forezi *et al.*, 2020).

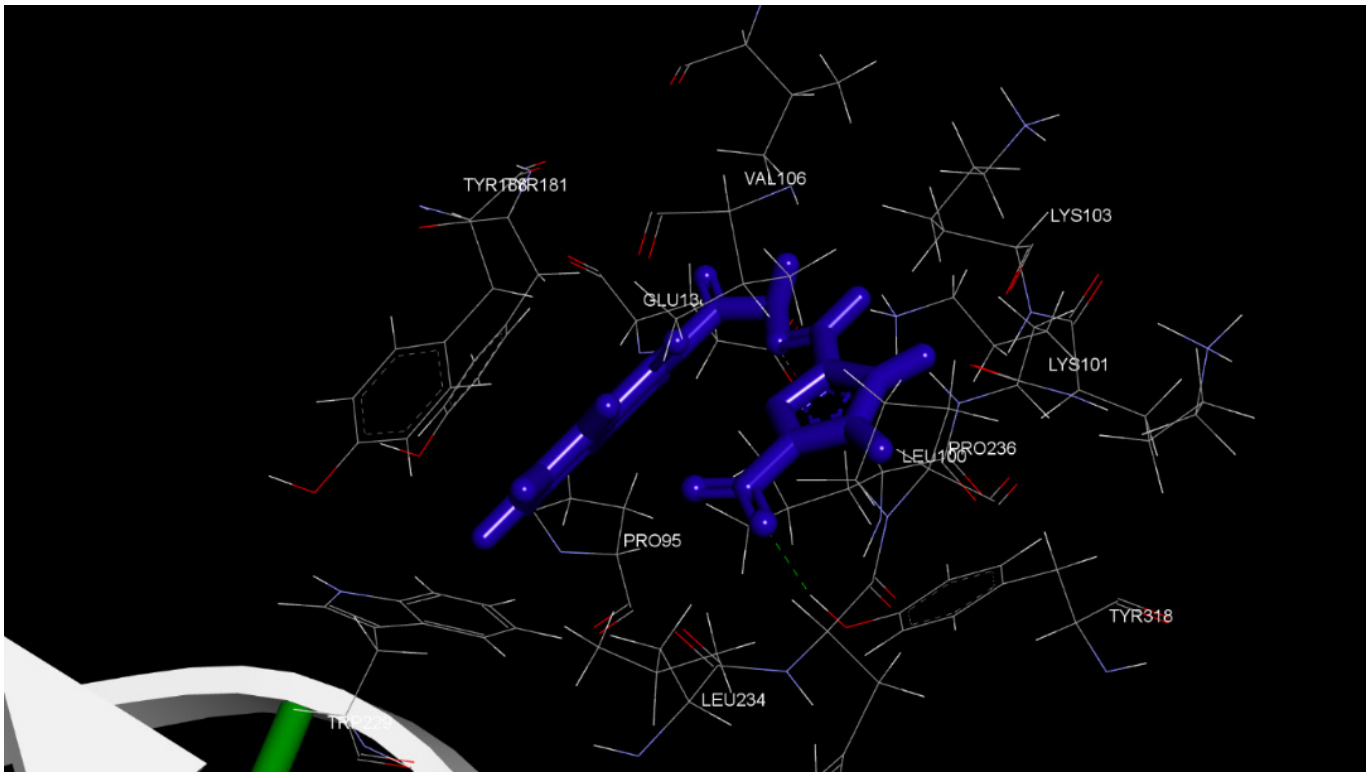

FIGURE 6- Nitro group of compound **3** (in blue) interacting by hydrogen bond (green dashed line) with Tyr318 residue from RT allosteric site.

In addition to the aforementioned steps, another docking was performed centering the binding site on the oxygen atom of the Trp239, set to 25 Å. This residue was chosen in order to obtain a binding site that encompasses the active site of the first stage and also the 95.23 Å^3 cavity of the second stage, allowing the assessment of all interactions observed in the first and second dockings. The algorithm shows the position with the lowest score (most stable), and for the three ligands the first 90 poses corroborates the previous results, indicating that the anchorage in the allosteric site was the preferred one among all the other cavities.

CONCLUSIONS

The anti-HIV-1 activity of oxoquinoline-acylhydrazone derivatives was investigated by molecular docking. The probable mechanism of action of these compounds is like NNRTIs inhibitors in the RT allosteric site. The results explain the HIV-1 activity observed for compounds **1**, **2** and **3,** and also indicates that **3** may be less susceptible to viral resistance since it interacts through hydrogen bond with residue Tyr318, for which mutations have not yet been described.

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