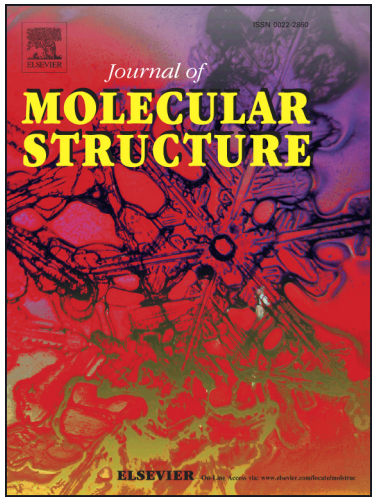


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# Docking of anti-HIV-1 Oxoquinoline-Acylhydrazone Derivatives as Potential HSV-1 DNA Polymerase Inhibitors

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**ABSTRACT**

Although there are many antiviral drugs available for the treatment of herpes simplex virus (HSV) infections, still the synthesis of new anti-HSV candidates is an important strategy to be pursued, due to the emergency of resistant HSV strains mainly in human immunodeficiency virus (HIV) co-infected patients. Some 1,4-dihydro-4-oxoquinolines, such as PNU-183792 (**1**), show a broad spectrum antiviral activity against human herpes viruses, inhibiting the viral DNA polymerase (POL) without affecting the human POLs. Thus, on an ongoing antiviral research project, our group has synthesized ribonucleosides containing the 1,4-dihydro-4-oxoquinoline (quinolone) heterocyclic moiety, such as the 6-Cl derivative (**2**), which is a dual antiviral agent (HSV-1 and HIV-1). Molecular dynamics simulations of the complexes of **1** and **2** with the HSV-1 POL suggest that structural modifications of **2** should increase its experimental anti-HSV-1 activity, since its ribosyl and carboxyl groups are highly hydrophilic to interact with a hydrophobic pocket of this enzyme. Therefore, in this work, comparative molecular docking simulations of **1**, **2** and three new synthesized oxoquinoline-acylhydrazone HIV-1 inhibitors (**3**-**5**), which do not contain those hydrophilic groups, were carried out, in order to access these modifications in the proposition of new potential anti-HSV-1 agents, but maintaining the anti-HIV-1 activity. Among the docked compounds, the oxoquinoline-acylhydrazone **3** is the best candidate for an anti-HSV-1 agent, and, in addition, it showed anti-HIV-1 activity ($EC_{50}$ = 3.4 ± 0.3 μM). Compounds **2** and **3** were used as templates in the design of four new oxoquinoline-acylhydrazones (**6**-**9**) as potential anti-HSV-1 agents to increase the antiviral activity of **2**. Among the docked compounds, oxoquinoline-acylhydrazone **7** was selected as the best candidate for further development of dual anti-HIV/HSV activity.

**Keywords:** acylhydrazone; dual antiviral agents; docking; HIV-1; HSV-1; quinolone; molecular modeling.

## 1. INTRODUCTION

In humans, the herpes simplex virus (HSV) causes mucocutaneous lesions, which are uncomfortable to the patient and may represent a life risk for immunocompromised individuals [1]. This virus exists in two forms: HSV-1 (associated to the oral disease) and HSV-2 (associated to the genital disease). At present, there is no cure for their effects, only treatments which can accelerate the scarring process of the formed vesicles or diminish the period between two consecutive disease manifestations.

HSV infections were one of the first diseases to be treated by means of antiviral agents. Among them, analogs of the natural nucleosides (e.g., guanosine) were introduced in the antiviral therapy, in 1960, targeting the viral DNA polymerase (POL), since it is a key enzyme in the viral replication cycle [2]. An alternative target for anti-herpes viral therapy is the viral helicase/primase complex [3], but in this work we will focus on the POL inhibitors. Guanosine analogs, such as aciclovir (ACV), ganciclovir, and penciclovir, act as viral POL inhibitors, in a competitive manner [3]. In fact, these nucleoside analogs are pro-drugs, which must be phosphorylated to the monophosphate form first by viral kinases, and then further phosphorylated to the triphosphate form, by cellular kinases before they become active inhibitors [4].

On the other hand, foscarnet (a pyrophosphate analogue), an antiviral drug available for the treatment of HSV and HIV (human immunodeficiency virus) resistant infections, directly inhibits POL by binding to a site on the HSV POL (EC: 2.7.7.7) or HIV reverse transcriptase (RT) (HIV RT has a POL domain, EC: 2.7.7.49) [5], and it is not dependent on activation (phosphorylation) by viral or cellular kinases [6]. Interesting, computational studies comparing the 3D structures of the polymerase domains of *Escherichia coli* POL I and HIV-1 RT, in spite of low sequence similarity, showed conserved residues that may represent a similar structure-function feature in all polymerases [7]. Moreover, considerable progress has been made in the development of potential drugs with dual (or multiple) antiviral activities, such as agents targeting HIV/HSV co-infection [8-12].

One of the limitations of the antiviral drugs available for the herpes treatment today is that they are active against only some of the eight human herpes viruses (HHV). However, some 1,4-dihydro-4-oxoquinolines, like PNU-183792 (**1**, Figure 1), inhibit six of the eight HHVs, showing a broad spectrum antiviral activity profile against human herpes viruses [13]. In addition, these non-nucleoside inhibitors [13-16] are selective because they inhibit the viral POLs without affecting the human POLs [4].

The broad spectrum antiviral activity of PNU-183792-like compounds can be explained by the fact that they seem to interact with a region within the domain III (residues 805-845) of the viral POL, which is extremely conserved in many herpes viruses [4], and they do not need phosphorylation by viral kinases [17]. Besides, they are also active against viruses resistant to ACV [13].

Although there are many antiviral drugs available for the treatment of HSV infections, the synthesis of new anti-HSV candidates is still an important strategy to be pursued [18, 19], due to the emergency of resistant HSV strains [20] mainly in HIV/HSV co-infected patients [21]. Thus, our group has synthesized ribonucleosides containing the 1,4-dihydro-4-oxoquinoline heterocyclic moiety, such as the 6-Cl substituted ribonucleoside (**2**, Figure 1), as new anti-HSV-1 candidates [17,22].

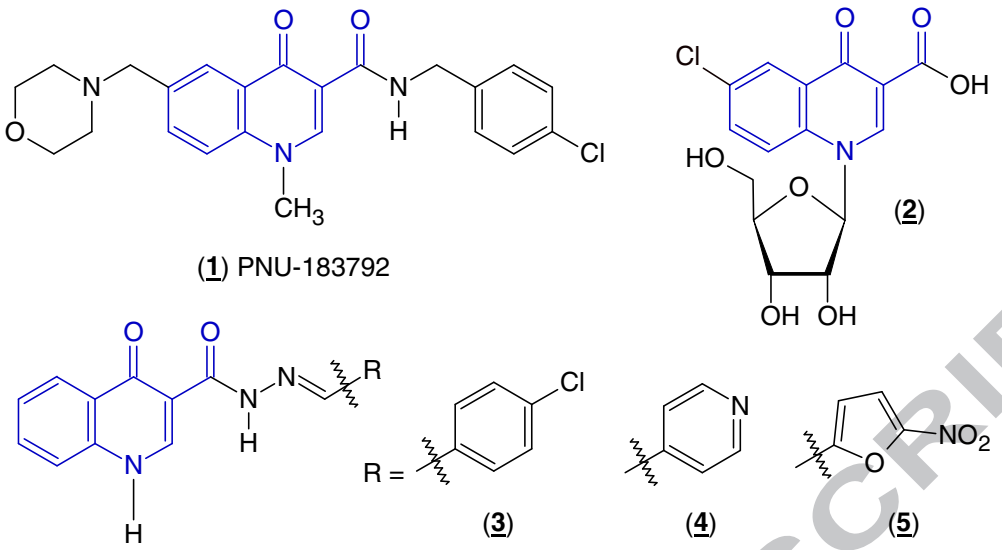


**Figure 1.** Chemical structures of PNU-183792 (**1**), [14] the 6-Cl substituted ribonucleoside (**2**), [17,22] and the oxoquinoline-acylhydrazones **3**, **4**, and **5** (in blue, the common 1,4-dihydro-4-oxoquinoline heterocyclic moiety).

The 6-Cl substituted ribonucleoside (**2**) inhibits HSV-1 replication, has low citotoxicity and high selective index [17,22]. It inhibits the HSV-1 POL activity but, differently from ACV, according to an uncompetitive mechanism, i.e., the inhibitor binds to the substrate (DNA) and enzyme (POL) complex [17]. Both the 6-Cl substituted ribonucleoside (**2**) and its aglycone moiety, which was also tested, inhibit the HSV-1 POL activity, showing that the main subunit responsible for the inhibition is the 6-Cl-1,4-dihydro-4-oxoquinoline ring (colored in blue in Figure 1) [17]. This result is in line with the previously mentioned studies of Wathen for the 1,4-dihydro-4-oxoquinolines [13].

However, the design and development of new drugs is a multistage and very expensive process, which must be properly planned and controlled [23]. In this context, molecular modeling is a useful tool for the analysis of the molecular details of compounds involved in biological processes, explaining the structure-activity relationship of molecules candidates to drug prototypes. Interesting docking and molecular dynamics studies have been developed focusing in a natural product targeting the HSV-1 DNA POL [24], but not including the substrate structure.

We have recently demonstrated that some structural modifications should increase the anti-HSV-1 activity of the ribonucleoside derivative mentioned earlier [25]. Besides, PNU-183792 is more active than **2**, according to experimental data from literature [7,22] (Table 1). Therefore, in this work, we use molecular modeling simulations, such as molecular docking, in order to rationalize these modifications and help our synthesis group to plan new potential anti-HSV-1 agents with anti-HIV activity.

**Table 1.** Anti-HSV-1 activity for compounds **1** [14] and **2** [22] compared to ACV.

| # | $EC_{50}$ (μM) | # | $EC_{50}$ (μM) |
|---|---|---|---|
| **1** | 3.3 | **2** | 1.5 |
| **ACV** | 3.9 | **ACV** | 1.25 |

The present study is based on the model proposed by Liu and co-workers [26]. According to this model, PNU-183792 (**1**, Figure 1), a compound which also contains a 1,4-dihydro-4-oxoquinoline heterocyclic moiety, binds to the double-stranded DNA (dsDNA) and POL complex, but does not bind to POL or to dsDNA alone [26]. This is in according to the mechanism of action proposed for the 6-Cl substituted ribonucleoside inhibitor (**2**, Figure 1). In contrast to other nucleoside inhibitors, such as ACV, and like PNU-183792, **2** is not dependent on viral or cellular phosphorylation events to generate an active form and does not form covalent complexes with the HSV POL [17].

In the Liu's model, PNU-183792 replaces the incoming nucleotide and dislocates the template base from the active site [26]. The oxoquinoline ring of PNU-183792 stacks against the primer-3'-end and the template, and also interacts with the active site residues of the HSV POL [26]. The *p*-Cl-phenyl group binds to a hydrophobic pocket lined by residues Phe820, Gln617, Gln618, and Val823 [26].

Our group has synthesized the oxoquinoline-acylhydrazones **3**, **4**, and **5** (Figure 1), which are active against HIV-1 in peripheral blood mononuclear cells (PBMC), according to the results in Section 3. Other acylhydrazones are reported as HIV-1 inhibitors, such as BBNH that inhibits both the RT POL and the RNaseH activities with similar potency ($IC_{50} \approx 3$ μM) [27]. In addition, the 6-Cl substituted ribonucleoside (**2**) is a dual antiviral agent, since it also inhibits HIV-1 replication targeting the RT enzyme [28].

Possibly, the inhibition mechanism of the oxoquinoline-acylhydrazones (**3**-**5**) is similar to that of the 6-Cl substituted ribonucleoside (**2**) and PNU-183792 (**1**) because the active aglycone moiety of **2** is common to all of them (Figure 1). Because the oxoquinoline-acylhydrazones (**3**-**5**), which shows activity against HIV-1, are structurally related to PNU-183792 (anti-HSV-1) and to the 6-Cl substituted ribonucleoside (**2**) (dual antiviral activity, anti-HSV-1 [17] and anti-HIV-1 [28]), these molecules are being used as a starting point for the proposition of new structures targeting the HSV POL enzyme, but maintaining the anti-HIV-1 activity.

## 2. MATERIALS AND METHODS

### *2.1. Molecular Modeling*

The structure of the HSV-1 POL was obtained from the Protein Data Bank [29] (PDB ID: 2GV9, chain B) [26]. Since some residues near to domain III are missing, this structure was reconstructed by comparative modeling, using the automated module of the Swiss-Model server [30]. The dsDNA-POL complex was created in the Swiss Pdb-Viewer program [31]. The 2GV9 structure, reconstructed by comparative modeling, was superposed to a theoretical model of the POL (PDB ID: 1B1F) [32].

The docking simulations were carried out using the residue module of the Surflex-Dock v.2.2 program [33,34]. The active site was generated selecting important residues from region III (i.e., Gln617, Gln618, Lys811, Asn815, Tyr818, Phe820, Val823, and Asp888), according to the Liu's model [26], with a radii of 3 Å related to them; from the template residues T6-T10; and from the primer residues P19-21. This selection gave better results, leading to a conformation and orientation for PNU-183792, used as a reference compound, closer to that proposed in the Liu's model.

Before docking, the structures of all the studied compounds, obtained by systematic conformational search in the Spartan program (Wavefunction, Inc.), were optimized using a B3LYP density functional with a 6-31G(d,p) basis set in the Gaussian 98 package [35]. Charges were calculated by fitting the electrostatic potential to a grid of points selected, according to the CHELPG scheme [36].

The docking studies were performed testing all tools available in the Surflex-Dock program to generate the binding mode according to the Liu's model, in which the residue-based approach showed the best performance [33,34]. In the case of PNU-183792, from the twenty structures generated by the docking output, we selected the one with the closest binding mode (conformation and orientation) to that proposed for this ligand in the Liu's model [26]. In the case of the other compounds, we selected those with the closest binding mode to the one selected for PNU-183792. For each selected pose, the ligand structure was optimized inside the active site of the protein, using the Surflex-Dock program. The partial atomic charges were recalculated according to the CHELPG scheme, using the Gaussian 98 program.

The final ligand-dsDNA-POL complexes obtained were submitted to the LPC/CSU server (http://ligin.weizmann.ac.il/cgi-bin/lpccsu/LpcCsu.cgi) [37] for automatic interactions analysis. The WebLab

ViewerLite program (Molecular Simulations, Inc.) was used for structure visualization.

In addition, the 2D structure of the compounds were analyzed on the OSIRIS Property Explorer program (Thomas Sander, Actelion Pharmaceuticals Ltd.; http://www.organic-chemistry.org/prog/peo/) in order to calculate the fragment-based drug-likeness score, which is based on the occurrence frequency of each fragment in the individual structure that are associated with drug-like (3300 traded drugs) and non-drug-like (15000 commercially available Fluka chemicals) databases.

*2.2. Synthesis*

The 3-acylhydrazone-oxoquinolines **3, 4**, and **5** were synthesized as previously described for our research group [38]. These analogues were prepared by reaction between the carbohydrazide and suitable aromatic aldehydes in ethanol, using hydrochloric acid as catalyst. The 5-nitrofuran derivative **5** was obtained by the condensation with commercially available (5-nitrofuran-2-yl)methylene diacetate in a mixture of ethanol and sulfuric acid 50%.

(**3**) (*E*)-4-oxo-*N'*-(4-chlorobenzylidene)-1,4-dihydroquinoline-3-carbohydrazide:

White solid. Yield 95%. Mp > 300ºC; $^{1}$H-NMR (500.00 MHz, DMSO-$d_6$) δ 13.32 (s, 1H, CONHN), 8.85 (s, 1H, H-2), 8.46 (s, 1H, H-1'), 8.31 (dd, 1H, J= 8.3; 1.1 Hz, H-5), 7.81 (ddd, 1H, J= 8.3; 8.3; 1.1 Hz, H-7), 7.78 (d, 2H, J= 8.3 Hz, H-4' and H-6'), 7.75 (d, 1H, J= 8.3 Hz, H-8), 7.54 (dd, 1H, J= 8.3; 8.3 Hz, H-6), 7.52 (d, 2H, J= 8.3 Hz, H-3' and H-7'); $^{13}$C-NMR (125.0 MHz, DMSO-$d_6$) δ 175.8 (C-4), 161.2 (C=ONHN), 146.1 (C-1'), 144.0 (C-2), 138.9 (C-8a), 134.2 (C-5'), 133.2 (C-2'), 132.7 (C-7), 128.6 (C-3' and C-7' or C-4' and C-6'), 128.5 (C-3' and C-7' or C-4' and C-6'), 125.7 (C-4a), 125.2 (C-5), 125.0 (C-6), 119.0 (C-8), 109.8 (C-3), Mass calcd. for $C_{17}H_{12}ClN_3O_2$ 325.0618; MS (ESI+) m/z found 326.0687 [M+H+].

(**4**) (*E*)-4-oxo-*N'*-(pyrid-4'-ylmethylene)-1,4-dihydroquinoline-3-carbohydrazide:

Yellow solid. Yield 90%. Mp > 300ºC; $^{1}$H-NMR (500.00 MHz, DMSO-$d_6$) δ 13.49 (s, 1H, CONHN), 8.87 (s, 1H, H-2), 8.67 (d, 2H, J= 6,0 Hz, H-4' and H-5'), 8.48 (s, 1H, H-1'), 8.31 (dd, 1H, J= 8.2; 1.1 Hz, H-5), 7.81 (ddd, 1H, J= 8.2; 8.2; 1.1 Hz, H-7), 7.76 (d, 1H, J= 8.2 Hz, H-8), 7.68 (d, 2H, J= 6.0 Hz, H-3'and H-6'), 7.54 (ddd, 1H, J= 8.2; 8.2; 1.1 Hz, H-6); $^{13}$C-NMR (125.0 MHz, DMSO-$d_6$) δ 175.9 (C-4), 161.6 (C=ONHN), 150.0 (C-4' and C-5'), 145.2 (C-1'), 144.4 (C-2), 141.5 (C-2'), 138.9 (C-8a), 132.9 (C-7), 125.8 (C-4a), 125.3 (C-5 and C-6), 120.9 (C-3' and C-6'), 119.1 (C-8), 109.7 (C-3); Mass calcd. for $C_{16}H_{12}N_4O_2$ 292.0960; MS (ESI+) m/z found 293.1035 [M+H+].

(**5**) (*E*)-4-oxo-*N'*-[(5-nitrofuran-2-yl)methylene]-1,4-dihydroquinoline-3-carbohydrazide:

Yellow solid. Yield 84%. Mp > 300ºC; $^{1}$H-NMR (500.00 MHz, DMSO-$d_6$) δ 13.50 (s, 1H, CONHN), 12.96 (d, 1H, J= 6.6 Hz, H-1), 8.86 (d, 1H, J= 6.6 Hz, H-2), 8.51 (s, 1H, H-1'), 8.31 (dd, 1H, J= 7.2; 1.1 Hz, H-5), 7.81 (ddd, 1H, J= 8.3; 7.2; 1.1 Hz, H-7), 7.76 (d, 1H, J= 3.8 Hz, H-4'), 7.75 (dd, 1H, J= 7.2; 1.1 Hz, H-8), 7.54 (ddd, 1H, J= 8.3; 7.2; 1.1 Hz, H-6), 7.17 (d, 1H, J= 3.8 Hz, H-3'); $^{13}$C-NMR (125.0 MHz, DMSO-$d_6$) δ 175.8 (C-4), 161.7 (C=ONHN), 151.8 (C-2' and C-5'), 144.4 (C-2), 138.8 (C-8a), 135.5 (C-1'), 132.9 (C-7), 125.7 (C-4a), 125.2 (C-5 and C-6), 119.0 (C-8), 114.3 (C-4'), 114.9 (C-3'), 109.5 (C-3), Mass calcd. for $C_{15}H_{10}N_4O_5$ 326.0651; MS (ESI+) m/z found 327.0718 [M+H+].

*2.3. Biological Tests*

*2.3.1. Cells and virus*

Peripheral blood mononuclear cells (PBMCs) from healthy human donors were obtained by density gradient centrifugation (Histopaque; Sigma, St. Louis, USA) from buffy coat preparations. PBMCs were resuspended in RPMI 1640 (LGC Bio, São Paulo, Brazil) supplemented with 10% FBS (Hyclone, Logan, USA), penicillin and streptomycin (CultiLab, São Paulo, Brazil), 2 mM glutamine and 10 mM HEPES (Sigma, St. Louis, USA) and stimulated with 5 mg/mL of phytohemagglutinin (PHA; Sigma, St. Louis, USA) for two to three days. They were further maintained in

culture medium containing 5 U/mL of recombinant human interleukin-2 (Sigma, St. Louis, USA). HIV-1 isolate Ba-L (R5-tropic, subtype B) [39] was used to infect cells. Virus isolates were prepared in PHA-activated PBMCs from healthy human donors.

*2.3.2. Screening of in vitro anti-HIV-1 activity*

This assay followed the procedures previously described [39] with minor modifications. Peripheral blood mononuclear cells (PBMCs) were initially exposed to viral suspensions containing 5-10 ng/mL of (Ba-L/R2) HIV-1 p24 Ag during 2-3 h. Cells were washed, resuspended in complete medium, plated in 96-well culture plates ($2.0 \times 10^5$ cells/well) in triplicate, and treated with different concentrations (μM) of compounds. After seven days at 37°C in 5% $CO_2$, viral replication was assessed by measuring the HIV-1 p24 Ag presence in culture supernatants by an ELISA capture assay (ZeptoMetrix Co., Buffalo, USA).

## 3. RESULTS AND DISCUSSION

The results for activity against HIV-1 in PBMC for the synthesized oxoquinoline-acylhydrazones **3**, **4**, and **5** (Figure 1), according to the biological tests described in Section 2.3, are shown in Table 2. The *p*-Cl-phenyl is the most potent (**3**, $EC_{50} = 3.5 \pm 0.3$ μM), followed by the 5-nitro-2-furanyl (**5**, $EC_{50} = 56 \pm 4.1$ μM) and the 4-pyridyl (**4**, $EC_{50} = 100 \pm 5.5$ μM).

**Table 2.** Results of *in vitro* antiviral activity against HIV-1 in peripheral blood mononuclear cells (PBMC) for the oxoquinoline-acylhydrazones **3**, **4**, and **5** (structures in Figure 1).

| # | % of inhibition at 50 μM | $EC_{50}$ (μM) |
|---|---|---|
| **3** | 99 ± 2.1 | 3.4 ± 0.3 |
| **4** | 10 ± 1.1 | 100 ± 5.5 |
| **5** | 40 ± 1.8 | 56 ± 4.1 |

In order to evaluate the potential binding mode of **3**, **4**, and **5** on the active site of the HSV-1 POL in complex with the dsDNA substrate, compounds **3**, **4**, and **5** along with **1** (PNU-183792) were docked into the dsDNA-POL complex, using the Surflex-Dock program. The best poses (Figure 2) are those in which the compounds show a binding mode (conformation and orientation) more similar to that proposed for PNU-183792 in the Liu's model, where the *p*-Cl-phenyl group of **1** points in the direction of Val823, a very important residue for the activity of compounds like PNU-183792 with the HSV-1 POL [4,25].

Figure 3 shows a close view of the superimposition of the docking output structures of compounds **3**, **4**, and **5** to PNU-183792 (**1**). According to Figure 3, compound **3** is found in an overall spatial arrangement more similar to that of PNU-183792 (including the *p*-Cl-phenyl group of both, **1** and **3**) than **4** or **5**, indicating that its anti-HSV-1 activity must be tested.

The ligand-protein interaction analysis of compounds **3**, **4**, **5**, and PNU-183792 (**1**), performed with the LPC server, showed that although the three compounds have thirteen interactions in common to that presented by PNU-183792, compound **3** is the one showing interactions with Thr887 (Table 3, Figure 4), near to Asp888 (Figure 4), considered an important residue from region III, according to the Liu's model [26]. Figure 4 shows compound **3** and PNU-183792 (**1**) in the active site with the morpholinyl ring of PNU-183792 (**1**) posed in accord to the Liu's model.

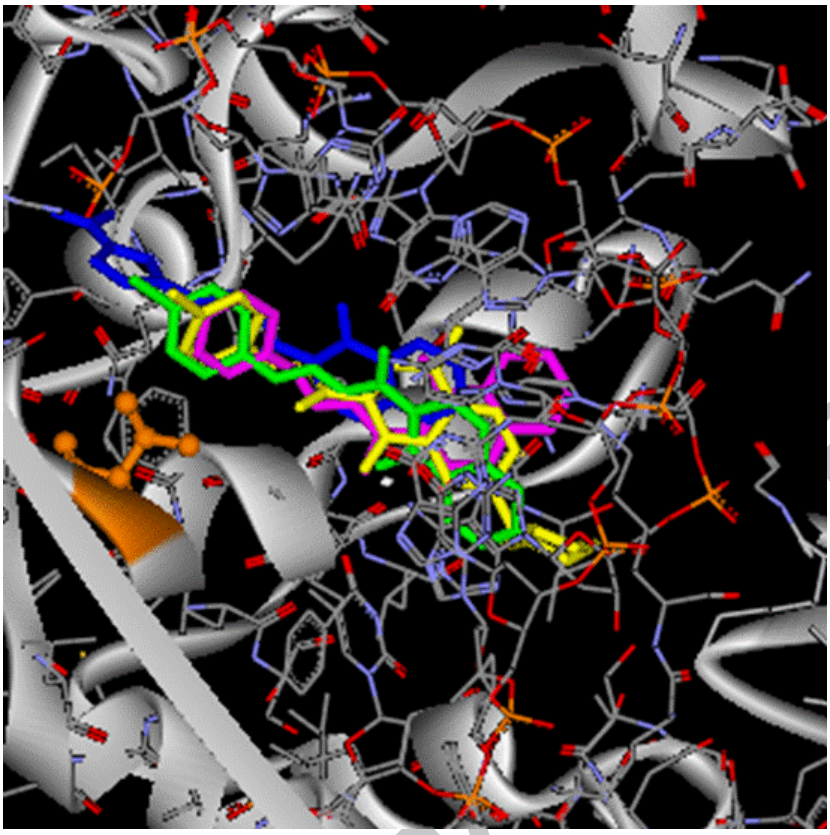

**Figure 2.** Best docking poses for compounds **3** (green), **4** (magenta), **5** (blue), and PNU-183792 (**1**) (yellow) close to Val823 (orange) into the HSV-1 DNA polymerase in complex with the substrate (dsDNA).

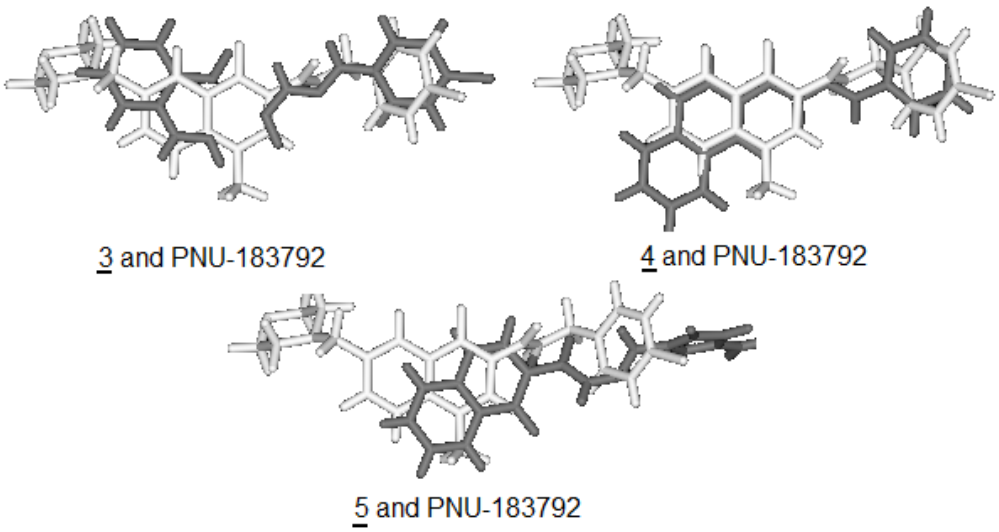


**Figure 3.** Superimposition of the docking output structures of compounds **3**, **4**, and **5** (gray) to PNU-183792 (**1**) (white).

**Table 3.** Interactions of compounds **3**, **4**, **5**, and PNU-183792 (**1**) with residues of HSV-1 DNA polymerase in complex with dsDNA. The protein residues considered most important by the Liu's model are in bold (2006), while P20 and P21 are the primer residues from DNA.

| Residue ID | 3 | 4 | 5 | 1 |
|---|---|---|---|---|
| Ile504 | X | X | X | X |
| Gly616 | | X | X | X |
| **Gln617** | X | X | X | X |
| **Gln618** | X | X | X | X |
| Ile619 | | | X | |
| Gln640 | X | | X | |
| Ser720 | | | | X |
| Pro723 | | | | X |
| Lys811 | | X | | X |
| Val812 | | | X | X |
| Cys814 | | | | X |
| **Asn815** | X | X | X | X |
| Ser816 | X | X | X | X |
| Tyr818 | X | X | | X |
| Gly819 | X | X | X | X |
| **Phe820** | X | X | X | X |
| **Val823** | X | X | X | X |
| His825 | X | | X | X |
| Gly826 | X | | X | |
| **Thr887** | X | | | X |
| P20 | X | X | X | X |
| P21 | X | X | X | X |
| Common to **1** | 13 | 13 | 13 | |

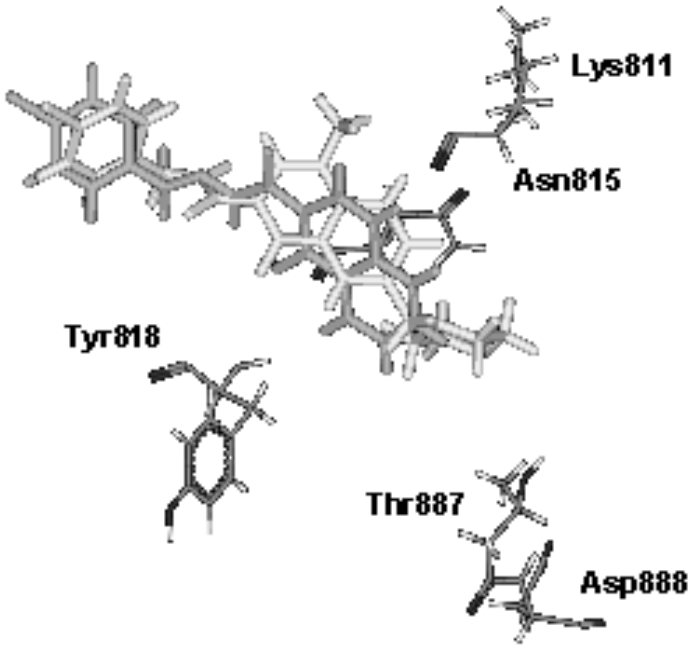


**Figure 4.** PNU-183792 (**1**) (white), compound **3** (gray), and some of the most important residues in the Liu's model (Asn815 and Thr887) and other residues close by (Lys811, Tyr818, and Asp888).

The docking results for compounds **1**, **3**, **4**, and **5** were used to propose four new oxoquinoline-acylhydrazone derivatives (**6**-**9**, Figure 5), using the concepts of structural simplification, molecular hybridization, and increasing conformational flexibility, considering compounds **2** (anti-HIV-1 and anti-HSV-1) and **3** (anti-HIV-1) as the reference structures, in order to increase interactions of the new proposed compounds with residues (from the enzyme and/or the primer/template DNA) in the POL active site.

All the proposed structures (**6**-**9**) will keep a chlorine atom at position 6 because they result from structural modifications earlier proposed for the 6-Cl substituted ribonucleoside (**2**). Structural simplification will be achieved by replacing the glycoside group of **2** (because it is highly polar, hydrophilic and it is not essential for anti-HSV-1 or anti-POL activities) by hydrogen (**6**) or alkyl groups (methyl, **7**; ethyl, **8**). Molecular hybridization will be achieved by merging the aglycone and *p*-Cl-benzylidene-hydrazine groups of **2** and **3**, respectively, in all proposed structures (**6**-**9**). The conformational flexibility of **7** (R = Me) (this compound has already been synthesized [38]) will be increased by reduction of the N=C bond in the design of **9**. Maybe, the free bond rotation of **9** could lead to a conformation and orientation more similar to that of PNU-183792 (**1**).

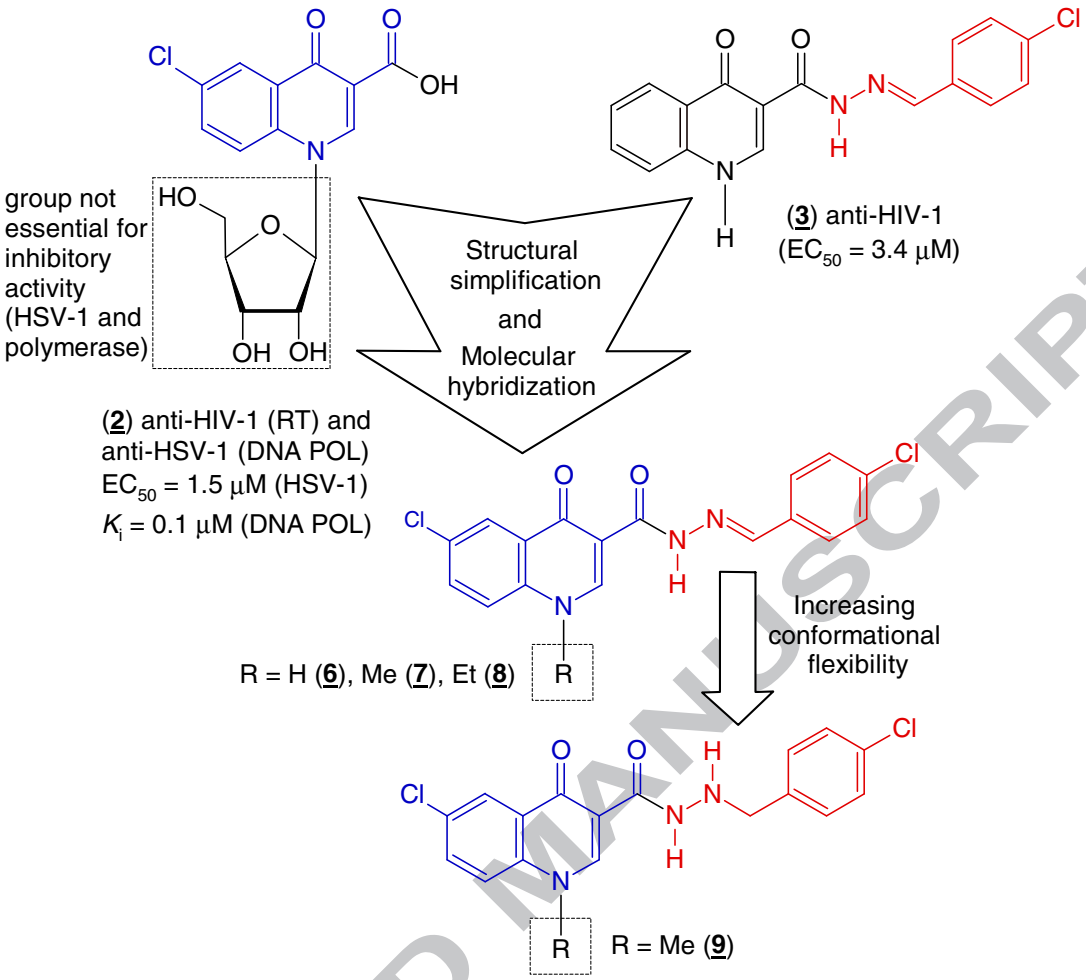


**Figure 5.** Structural modifications proposed for the 6-Cl substituted ribonucleoside (**2**) and compound **3** in the design of the new four oxoquinoline-acylhydrazone derivatives **6** (R=H), **7** (R=Me), **8** (R=Et), and **9** (R=Me).

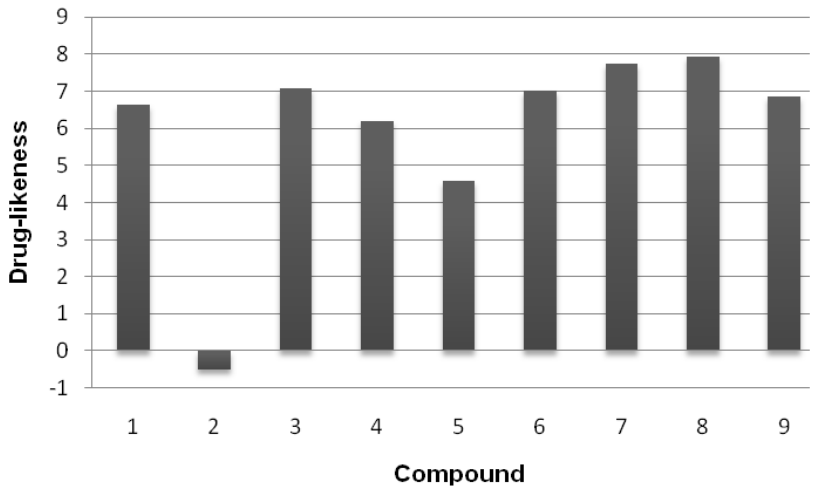


**Figure 6.** Drug-likeness scores (OSIRIS Property Explorer) for PNU-183792 (**1**), 6-Cl substituted ribonucleoside (**2**), oxoquinoline-acylhydrazones (**3**, **4**, and **5**) and the new proposed oxoquinoline-acylhydrazones (**6**, **7**, **8**, and **9**).

The fragment based drug-likeness profile of all compounds (**1**-**9**) performed on the OSIRIS Property Explorer program is shown in Figure 6. Among the synthesized oxoquinoline-acylhydrazones (**3**-**5**), only **3** (the most potent compound against HIV-1, Table 2) has a drug-likeness score higher than that of PNU-183792 (**1**) (Figure 6). Among the new proposed oxoquinoline-acylhydrazones (**6**-**9**), **7** and **8** showed the greatest drug-likeness, even greater than PNU-183792 (**1**) and **3**, indicating that they are good drug candidates. The 6-Cl substituted ribonucleoside (**2**) was the only compound that showed a negative drug-likeness score (Figure 6), indicating that, in fact, the proposed structural modifications are interesting in order to turn it into a better drug candidate.

The docking poses for the new proposed oxoquinoline-acylhydrazones **6**, **7**, **8**, and **9** with dsDNA-POL superposed to PNU-183792 (**1**) are shown in Figure 7. Proposed derivatives **6** (R=H) and **7** (R=Me) had a spatial arrangement closer to that obtained in the docking result of PNU-183792, while **8** (R=Et) had its orientation completely inverted, probably because the replacement of the methyl by ethyl group imposed a steric hindrance. Compound **9** (the reduced analog of **7**, R=Me) showed two possible poses, probably due to its higher conformational freedom: one with its methyl group superposed on the morpholinyl ring of PNU-183792 (pose **9a**) and the other with its methyl group superposed on the methyl group of PNU-183792 (pose **9b**).

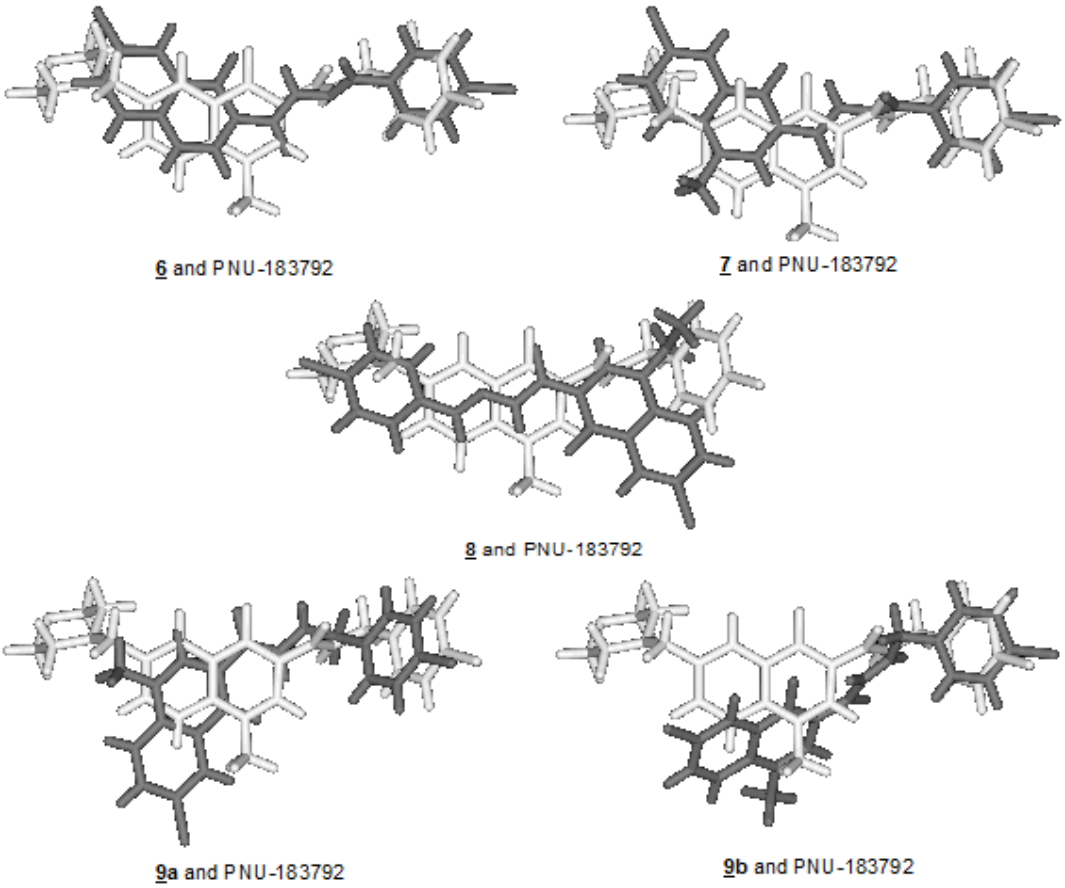


**Figure 7.** Docking results for the new proposed oxoquinoline-acylhydrazones (**6**, **7**, **8,** and **9**, in gray) with HSV-1 DNA polymerase superposed to PNU-183792 (**1**, in white).

The analysis of the interactions between the docking output structures and the POL (Table 4) show that the compounds **6** and **7** are the ones for which interactions as that of PNU-183792 (**1**) increased the most. They also keep the same interactions made by compound **3**. Compound **6** gained five interactions (compared to compound **3**) with POL residues, two of which are common to PNU-183792, whereas compound **7** gained four interactions, three of which are common to PNU-183792. As mentioned before, compound **8** shows a completely inverted orientation compared to PNU-183792, while compound **9** (poses **a** and **b**) shows a superimposition to PNU-183792 not so good as those of compounds **6** and **7** (Figure 7).

**Table 4.** Interactions of the new proposed oxoquinoline-acylhydrazones (**6**, **7**, **8** and **9**) and PNU-183792 (**1**) with residues of HSV-1 DNA polymerase in complex with dsDNA (T6 is a template and P18, P20, and P21 are primer residues from DNA; X* and *** represent interactions gained and lost, respectively, compared to compound **3**).

| Residue ID | 6 | 7 | 8 | 9a | 9b | 1 |
|---|---|---|---|---|---|---|
| **Ile504** | X | X | X | X | X | X |
| **Lys534** | | | | | X* | |
| **Gly616** | X* | X* | X* | | | X |
| **Gln617** | X | X | X | X | X | X |
| **Gln618** | X | X | X | X | X | X |
| **Ile619** | | | | | | |
| **Gln640** | X | X | *** | X | *** | |
| **Ser720** | | | | | | X |
| **Tyr722** | X* | X* | | | | |
| **Pro723** | | X* | | | | X |
| **Lys811** | | | | X* | X* | X |
| **Val812** | X* | | | X* | X* | X |
| **Cys814** | | X* | | | | X |
| **Asn815** | X | X | X | X | X | X |
| **Ser816** | X | X | X | X | X | X |
| **Tyr818** | X | X | X | *** | X | X |
| **Gly819** | X | X | X | X | X | X |
| **Phe820** | X | X | X | X | X | X |
| **Val823** | X | X | X | X | X | X |
| **His825** | X | X | *** | X | X | X |
| **Gly826** | X | X | *** | X | *** | |
| **Leu827** | X* | | | | | |
| **Thr887** | X | X | X | X | *** | X |
| **T6** | X* | | | | | |
| **P18** | | | X* | | | |
| **P20** | X | X | X | X | X | X |
| **P21** | X | X | X | X | X | X |
| **Common to 1** | 15 | 16 | 13 | 14 | 14 | |

Accordingly, we are proposing the synthesis of the new proposed oxoquinoline-acylhydrazones **7** (Figure 5), which has a high drug-likeness score (Figure 6) and shows interaction with residues Gly616, Tyr722, Pro723, and Cys814. These interactions are important because they are maintained by PNU-183792 (**1**) during 8 ns of molecular dynamics simulations [25]. Besides, the methyl group in **7** makes it more interesting than structure **6**, due to a "push-pull" effect which seems to be important in the molecular dynamics simulation studies [25].

## 4. CONCLUSIONS

According to our docking studies, oxoquinoline-acylhydrazone **3**, the most potent compound against HIV-1 ($EC_{50}$ = 3.4 ± 0.3 μM), is a good candidate to be an anti-HSV-1 agent. In order to increase the anti-HSV-1 activity of the 6-Cl substituted ribonucleoside **2**, but maintaining its anti-HIV-1 activity, we propose four new oxoquinoline-acylhydrazones (**6**-**9**) as good candidates to anti-HSV-1 agents. Among them, **7** was selected as the best candidate to synthesis and further development of dual anti-HIV/HSV agents.

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JDY held a graduate fellowship from CAPES and FCS from FAPERJ. RBA has a fellowship from FAPERJ. PRS, MGA, MCBVS, RBA and VFF have research fellowships from the CNPq. We would also like to thank FAPERJ for funding support.

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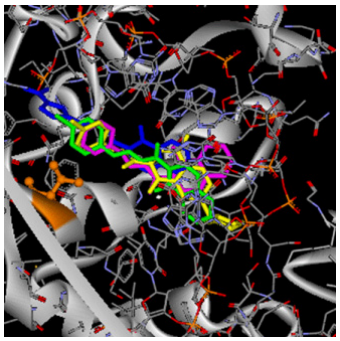
Graphical abstract

In this work, we use molecular modeling simulations, such as molecular docking, in order to rationalize structural modifications which should increase the anti-HSV-1 activity of a ribonucleoside containing the 1,4-dihydro-4-oxoquinoline heterocyclic moiety.



## Highlights

. Compound **3** is a good candidate to be an anti-HSV-1 agent.

. Four compounds were proposed as good candidates to anti-HSV-1 agents.

. Compound **7** was selected as the best candidate to synthesis and further studies.