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# Advances in the Development of Pyridinone Derivatives as Non-Nucleoside Reverse Transcriptase Inhibitors

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## ABSTRACT

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are part of a structurally diverse family with distinct features attractive for the treatment of AIDS that continue to be a major health problem. NNRTIs are highly selective to HIV-1 reverse transcriptase and have, in general, high selectivity and lower toxicity than other anti-AIDS drugs. However, non-optimal pharmaceutical properties and resistance mutations highlight the need to continue identifying and developing novel NNRTIs. Derivatives of pyridin-2(1*H*)-one are promising NNRTs under pre-clinical development. Herein, we survey the evolution of the pyridin-2(1*H*)-one class over the past 25 years: from the first generation of compounds with weak inhibitory activities against mutant strains to advanced generations with improved activity profile against clinically relevant mutants. Crystallographic structures, structure-based and ligand-based computational analysis, and medicinal chemistry efforts have worked in synergy to develop this important chemical class. We also discuss recent trends and future directions that can further improve the activity of pyridin-2(1*H*)-ones against clinically relevant mutant strains.

**Key words:** AIDS; Antiviral; Chemotherapy; Computer-aided drug design; HIV-1; Inhibitor; Medicinal chemistry; Reverse transcriptase.

**List of abbreviations:** 3D-QSAR, three-dimensional quantitative structure-activity relationships; AIDS, Acquired Immune Deficiency Syndrome; CoMFA, Comparative Molecular Field Analysis; CoMSIA, Comparative Molecular Similarity Analysis; FDA, Food and Drug Administration; HAART, highly active antiretroviral therapy; HIV, Human Immunodeficiency Virus; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; PDB, Protein Data Bank; RT, reverse transcriptase; SAR, structure-activity relationships; SI, selectivity index; WT, wild-type.

## 1. INTRODUCTION

Acquired Immune Deficiency Syndrome (AIDS) continues to be a major health problem. According to the World and Health Organization WHO, in 2014 there were 36.9 million people living with the human immunodeficiency virus (HIV). In the same year, there were 2.0 million of new infections and 1.2 million people died from AIDS.<sup>1</sup> Drugs available today for the treatment of HIV-1 infections can be classified in the following classes: reverse transcriptase (RT) inhibitors: nucleoside (nucleotide) (NRTIs) and non-nucleoside (NNRTIs); HIV protease inhibitors, integrase inhibitors, a fusion inhibitor (to prevent the fusion of the viral envelope with the host-cell membrane), and a C-C chemokine receptor type five (CCR5) inhibitor (to block the interaction of the virus with this receptor at the host cell).<sup>2</sup> These drugs are administered through the Highly Active Antiretroviral Therapy or HAART in various combinations and administration schedules. Current treatments require the combination of at least two or three active drugs from at least two different classes. For instance, Atripla and Complera are combinations of two NRT inhibitors and one NNRTI. HAART has been helpful to reduce the viral loads in patients, reducing the incidence of opportunistic infections and death in AIDS patients. However, treatments eventually fail due to the emergence of resistance. Resistance is primarily associated with the development of mutations in RT, integrase, and HIV protease.

In this paper we survey the progress on the development of NNRTIs with a special emphasis on the pyridine-2(1*H*)-one chemical class. Pyridinone derivatives were one of the first chemical classes to be investigated as anti-HIV compounds. Similar to other NNRTIs, due to the emergence of resistance, several groups around the globe have been working on the optimization of pyridinone derivatives using a wide range of experimental and computational tools. This manuscript is organized in five sections. After this introduction, a brief overview of the structure of RT is presented in Section 2. Section 3 discusses the current status of NNRTIs including the major strategies that are being pursued to overcome resistance. It is also presented an overview of the chemical classes that are being developed. Section 4 describes the development and current status of pyridine-2(1*H*)-one derivatives as NNRTIs. Contributions of computational methods to develop pyridin-2(1*H*)-one derivatives and current trends to further develop this chemical class are also discussed. Finally, Concluding remarks are presented in section 5.

## 2. REVERSE TRANSCRIPTASE (RT)

HIV-1 RT is a non-symmetric heterodimer that has a p66 subunit (with 560 amino acid residues) and a p51 subunit (with 440 amino acid residues). At the time of writing (November 2015) there were more than 200 crystallographic structures of HIV RT in the Protein Data Bank (PDB).<sup>3</sup> These structures include wild-type (WT) and mutant enzymes; for more detail see Table S1 in the Supporting Information. Interestingly, 197 of these structures were co-crystallized with a ligand, being nevirapine and rilpivirine the most common ligands (with 21 and 14 structures, respectively). On the other hand, only 27 structures were crystallized in the free state or in complex with DNA.

A three-dimensional (3D) structure of RT is shown in Figure 1. The 3D structure of the p66 unit has been compared to a right hand with a “fingers”, “palm” and “thumb” domain.<sup>4</sup> In this analogy, the “fingers” correspond to amino acids 1-85 and 118-155; the “palm” is associated with amino acid residues 86-117 and 156-237; and the “thumb” with amino acid residues 238-318. The palm domain

includes the polymerase active site (aspartic acid residues 110, 185, and 186 marked in red in Figure 1).<sup>2</sup> The palm domain also includes the binding (allosteric) site of the NNRTIs that is located about 10Å away from the catalytic site (for example, see yellow region in Figure 1). This allosteric site is hydrophobic in nature. It is comprised of two residues in the p51 subunit: Ile135 and Glu138; and the following residues in the p66 subunit: Tyr181, Tyr188, Phe227, Trp229, and Try232 (aromatic); Pro59, Leu100, Val106, Val179, Leu234, and Pro236 (hydrophobic) and Lys101, Lys103, Ser105, Asp132, and Glu224 (hydrophilic). This binding site is non-existing in the unbound RT but it is created upon binding of a NNRTI. Upon binding of a NNRTI, the sidechains of Tyr181 and Tyr188 rotates toward the catalytic site. In turn, this rotation shifts the position of the three catalytic aspartic acid residues by ~2Å. These conformational modifications are thought to be the basis of the inhibitory effect against the enzyme.<sup>2</sup> Different techniques continue to be applied to elucidate the role and movement of amino acid residues upon binding of NNRTIs. For instance, a recent NMR study identified a correlation between <sup>13</sup>C shifts of the M230 resonance of HIV-RT with the activity of nevirapine, delavirdine, efavirenz, dapivirine, etravirine, and rilpivirine.<sup>5</sup>

Despite the broad availability of structural information for RT, docking-based virtual screening has only led to few novel lead compounds with improved potency compared to known compounds. This fact has been associated, at least in part, with the induced fit mechanism that uses the NNRTIs to bind RT.<sup>6</sup> However, there are examples that clearly show that docking-based virtual screening is useful to uncover compounds with improved activity against mutant strains.<sup>7,8</sup>

<Insert Figure 1 here>

### 3. NON-NUCLEOSIDE HIV-1 RT INHIBITORS

Contrary to NRTIs, NNRTIs are highly selective toward the HIV-1 RT enzyme and therefore, exert minimum side-effects. Indeed, NNRTIs continue to be a major component of anti-HIV therapy.<sup>2</sup>

### 3.1 NNRTIs approved for clinical use

There are five NNRTIs approved for clinical use which can be grouped in three generations based on their activity profile and approval (Figure 2A). The first generation: nevirapine (approved by the USA Food and Drug Administration, FDA in 1996), delavirdine (approved in 1997) and efavirenz (approved in 1998). Nevirapine and efavirenz continue to be an important part of HAART, in particular efavirenz which is the most extensively used NNRTI. Overall, delavirdine is much less used because of its poor pharmacokinetics. The first generation of NNRTIs is characterized by a low genetic barrier to resistance (*i.e.* only one mutation is enough to reduce the efficacy of these compounds), hence they are usually co-administered with at least two other non-NNRTI antiretroviral drugs. Etravirine, also administered in combination with other antiretroviral drugs, was the first next generation NNRTI to be approved by the FDA in 2008. This drug was characterized by retaining activity against mutant strains.<sup>2</sup> Rilpivirine was the next drug approved in 2011 by the FDA for clinical use. This drug belongs to the same chemical class of etravirine (Figure 2A) but has a better activity profile against mutant strains (*vide infra*). Efavirenz and rilpivirine are part of the combination therapies Atripla and Complera which are amongst the most effective therapies. However, the rapid mutation of the virus, side effects, and not optimal pharmacological properties of current NNRTIs prompts the need to develop novel NNRTIs. For instance, hepatotoxicity and severe rash are associated with the use of nevirapine. Efavirenz is associated with CNS side effects that are sometimes difficult to overcome, especially in the first days of administration.<sup>2</sup> Hypersensitivity reactions or other adverse effects have been observed with the treatment of second generation NNRTIs.<sup>6</sup>

<Insert Figure 2 here>

### 3.2 The challenge: overcoming drug resistance

As commented by Das *et al.*, it is desirable that a NNRTI blocks the replication of resistant viral strains and preserve the potency for extended periods of time.<sup>9</sup> Mutations associated with resistance to

NNRTIs are located within the non-nucleoside binding pocket (as mentioned above, this binding pocket is approximately 10 Å away from the nucleoside binding site). As recently reviewed, almost all of the residues in the binding pocket can mutate and the majority of them can confer resistance to NNRTIs.<sup>10</sup> Examples of the mutations observed are L100I, K101E, V106A, K103N, V179D, Y181C, Y188L, G190A, and E138K in p51. Two of the most clinically relevant mutations are K103N and Y181C. In particular, the K103N mutation gives an almost uniform level of cross-resistance to most NNRTIs. Béthune published a rich review of common mutations associated with NNRTIs resistance and their impact on the susceptibility of HIV-1 to NNRTIs.<sup>2</sup>

Mutations either eliminate key (pharmacophoric) interactions with the small-molecule inhibitor, or affect the size/shape of the binding pocket preventing the inhibitor from a suitable binding. Specifically, mutations Y181C or Y188L cause loss of aromatic ring stacking interactions; mutations L100I or G190A/S are associated with steric hindrance, and mutations V106A or V179D modify hydrophobic interactions.<sup>10</sup> Also, it has been commented that the K103N mutation might induce resistance by stabilizing the unbound form of RT.<sup>11</sup>

During the design and development of NNRTs with improved activity against resistance mutants it has been observed that conformational flexibility and positional adaptability play an important role to retain activity against mutant strains.<sup>9</sup> For instance, during the development of etravirine (Figure 2A), a diarylpyrimidine or DAPY derivative, crystallographic structures and molecular modeling studies revealed that the torsional flexibility of certain chemical bonds in the structure strategically located, plus the ability of the molecule to adapt its position and orientation, are associated with a retained potency against a wide range of drug-resistant HIV-RTs.<sup>9</sup> This is exemplified by the high-resolution structures of the analogue rilpivirine (Figure 2A) bound to WT, and double mutants K103N/Y181C and L100I/K103N HIV-RT.<sup>12</sup> Rilpivirine inhibits K103N, Y181C, and K103N/Y181C RT mutants at an  $EC_{50} < 1$  nM. Authors of that work concluded that the crystallographic structures (*i.e.*, PDB IDs:

2ZD1, 3BGR, and 2ZE2, respectively) gave an understanding of the role of the flexibility of the inhibitor to compensate for drug-resistance mutations.<sup>12</sup>

### 3.3 Compound classes under (pre-clinical and clinical) development

The chemical structures of NNRTIs are highly diverse comprising more than 50 families of molecules. Li *et al.* published an excellent paper with a recent review of NNRTIs<sup>6</sup> highlighting the chemical classes discussed in the patent literature from 2011 to 2014. In addition to drugs approved for clinical use (*vide supra*, Figure 2A) other compounds are in clinical development such as the triazole RDEA806 and the phosphoindole IDX899 (GSK2248761) (Figure 2B). The development of the pyrazole lersivirine (UK-453061) was discontinued in 2013.

Bicyclic arylaminoazines are examples of one of the compound classes under (pre-clinical) development.<sup>13</sup> Extensive structural-activity relationships (SAR) studies coupled with structure-based analysis have recently led to two compounds with sub-10 nM potency against WT and clinically relevant viral variants of HIV-1 (compound **1** in Figure 2B).<sup>14</sup> The compounds also showed acceptable cytotoxicity and solubility better than rilpivirine. Of note, a crystallographic structure of **1** is available (ligand query 639 in PDB ID: 5C25).

## 4. PROGRESS ON THE DEVELOPMENT OF PYRIDIN-2(1H)-ONE DERIVATIVES

Among the chemically diverse NNRTIs, including the classes discussed by Li *et al.* (*vide supra*),<sup>6</sup> pyridin-2(1H)-ones represent a promising class. This chemical family has evolved from typical NNRTIs that were initially inactive against mutant strains, to advanced generations with improved activity profile against clinically relevant mutants. The evolution of pyridin-2(1H)-one chemical class as NNRTIs is discussed in this section. Figure 3 shows the chemical structures of representative molecules discussed. Note that, in contrast to the patent review paper published by Li *et al.*, the current paper is focused on one major class, pyridinone derivatives. As discussed below, the present review

spans nearly 25 years of development of this major structural class of NNRTIs since the first reports in 1991 to the time of writing this manuscript. In addition, herein we discuss not only patent literature but scientific journals as well.

<Insert Figure 3 here>

#### 4.1. The beginnings: Merck pyridinones

Pyridin-2(*1H*)-one derivatives as NNRTIs were first reported in 1991.<sup>15</sup> These compounds were identified from a screening program at Merck. One of the first potent and selective HIV-1 RT inhibitor of this class had a phthalidimide ring and the molecule (L-345-516) was hydrolytically unstable. An extensive optimization program led to the development of compounds that moved into clinical trials.<sup>16-18</sup> Representative compounds are the Merck pyridinones L-697,661, L-697-639, and L-696-229 (Figure 3). However, the emergence of resistance hampered their further development. In particular, the fast emergence of drug-resistant HIV strains containing the Y181C, Y188L, and K103N mutations in RT ultimately led to the abandonment of the clinical development of the Merck pyridinones. However, as discussed in detail in the next sections, the pyridin-2(*1H*)-one ring remained as basic core for further optimization.

As part of the early optimization of the Merck pyridinones an important amount of SAR information was accumulated. For instance, for this series of Merck pyridinones the best combination of alkyl groups at the C-5 and C-6 positions was 5-ethyl/6-methyl. This optimal combination for these series was taken as a starting point to develop new derivatives with improved resistance profile.

##### 4.1.1 Docking studies with Merck pyridinones

During the early development of pyridin-2(*1H*)-one derivatives as NNRTIs computational simulations were conducted to aid in the rationalization of the SAR at the structural level. Of note, there were not available crystallographic structures of RT bound to this class of inhibitors. Flexible docking of Merck pyridinones with a series of crystallographic structures of RT co-crystallized with other NNRTIs<sup>19</sup>

indicated that the pyridinones adopt the classical butterfly-like conformation of several other NNRTIs. The docking models also indicated that the lactam NH group forms the “signature” hydrogen bond with Lys101 that many NNRTIs have. Results also suggested that the benzoxazol ring of compounds such as L-697,661 and L-696,229 (Figure 3) made aromatic interactions with Tyr181. Therefore, the docking models provided a structure-based rationale to replace the benzoxazol ring with other structural moiety as further elaborated in the next section.<sup>19</sup>

#### 4.2. Early development of pyridin-2(1*H*)-one hybrids with other NNRTIs

In 1995, the synthesis of hybrids with HEPT and analogues such as TNK651<sup>20</sup> (Figure 2B) was published as one of the first modifications of the Merck pyridinones to improve their activity profile against drug-resistant mutants. The hybrid design was based on the comparison and replacement of the pyridin-2(1*H*)-one scaffold with the central pyrimidine ring of HEPT; if the 3-4 NH lactam of the pyrimidine ring of TNK651 (Figure 2B) is overlapped with the NH lactam of the pyridin-2(1*H*)-one ring (Figure 3A), the C-6 benzyl of TNK651 (or 6-phenylthio of HEPT) is equivalent to the C-4 position of the pyridin-2(1*H*)-one ring (Figure 3). Notably, crystallographic structures of RT in complex with TNK651 revealed the significance of the C-6 benzyl group to enhance the interactions with the binding site.<sup>20</sup> This combination gave rise to the new sub-class 4-(arylthio)-pyridin-2(1*H*)-ones.<sup>21</sup> Further SAR investigation led in 2000 to the development of 4-benzyl-pyridin-2(1*H*)-ones.<sup>22</sup>

One of the first most promising compounds in the 4-benzyl subclass was compound **2** (Figure 3 and Table 1). In addition to have a 3,5-dimethyl benzyl group at C-4, this molecule is characterized by having a dimethyl amino group at C-3, an ethyl group at C-5 of the pyridinone ring, and methyl group at C-6. The 4-benzyl-pyridin-2(1*H*)-one **2** showed a strong inhibition of recombinant HIV-1 RT and nanomolar inhibition of an HIV-1 resistant strain to nevirapine.<sup>22</sup> Of note, the optimal 5-ethyl/6-methyl combination at the C-5 and C-6 positions of the Merck pyridinones (*vide supra*) largely influenced the development of the first series of pyridin-2(1*H*)-one/HEPT hybrids. As discussed below, the additional but independent improvement of HEPT that gave rise to TNK651<sup>20</sup> guided the design of other pyridin-

2(1*H*)-one/HEPT hybrids with different substituents, in particular at C-6 (*vide infra*). Interestingly, during the early development of 4-(aryltio)-pyridin-2(1*H*)-ones<sup>21</sup> and 4-benzyl- pyridin-2(1*H*)-ones,<sup>22</sup> no docking studies were reported with crystallographic structures of RT available at that time.

<Insert Table 1 here>

#### 4.3. Further development of pyridin-2(1*H*)-one/NNRTIs hybrids: crystallographic structures

During the next few years Benjahad *et al.* continued the exploration of the SAR of 4-aryltiopyridinones and benzylpyridinones.<sup>23, 24</sup> One of the compounds that emerged from such extensive SAR exploration was the benzylpyridinone R157208<sup>24</sup> (Figure 3). A particular structural feature of this compound is an N-3 motif. The *in vitro* activity profile of this compound is summarized in Table 1. An extensive activity profile across double mutants is fully detailed in Benjahad *et al.*<sup>24</sup> R157208 showed an acceptable activity profile against mutant strains relevant in the clinic, comparable to that of efavirenz. In the SAR study other analogues related to the structure of R157208, however, showed an overall better profile, comparable to that of the parent molecule. In that work was concluded that substitution at the N-3 is very sensitive to the activity.<sup>24</sup> In particular, augmenting the steric bulk at this motif has a negative influence on the activity.

A notable contribution of that extensive SAR effort was the generation of a crystallographic structure of R157208 in complex with WT RT first published in Benjahad *et al.*<sup>24</sup> and then further analyzed in Himmel *et al.*<sup>25</sup> The co-crystal structure revealed that the 4-benzyl substituent make interactions with the hydrophobic pocket formed by residues Tyr188 and Trp229. The group at C-3, namely, the 3-(2-methoxyethyl)methylamino, makes interactions primarily with Tyr181.

Almost simultaneously that the crystallographic structure of HIV-1 RT complexed with R157208 was published<sup>24</sup> Himmel *et al.* released two additional crystallographic structures of HIV-1 RT bound to the pyridin-2(1*H*)-ones R165481 and R221239 (Figure 3).<sup>25</sup> These two molecules belong to the 3-iodo-4-aryloxy pyridinone -IOPY- subclass.<sup>26</sup> Interestingly, one of the previous lead compounds from the 3-iodo-4-aryloxy pyridinone sub-class, compound **3** (Figure 3 and Table 1), was initially developed

with the goal of developing a synthetic route to introduce a broad variety of substituents at C-3. Unexpectedly, compound **3** became a lead compound itself with a promising activity profile against several drug-resistant mutant strains as shown in Table 1.<sup>26</sup>

Table 2 summarizes the crystallographic information available for pyridin-2(1*H*)-ones bound to HIV-1 RT. Other selected structures are shown for comparison.

R165481 and R221239 are characterized by an iodine substitution at C-3, in contrast to the 3-(2-methoxyethyl)methylamino group of R157208 (Figure 3). Similar to R157208, compounds R165481 and R221239 have nanomolar or sub-nanomolar activity with the WT RT but different activities with drug-resistant strains. Table 1 summarizes the activity profile against WT and representative mutant strains. Further activity information is presented at Himmel *et al.*<sup>25</sup> Of note, the crystallographic structures of the two co-crystal compounds that have an iodine atom at C-3 do not make extensive contacts with Tyr181. Indeed, the strong activity profile of R165481 and R221239 against mutants can be attributed in part to the interaction of the C-3 iodine atom with the main-chain carbonyl oxygen of Tyr188. It was also concluded in that study that the acrylonitrile substituent on the C-4 phenoxy ring of R165481 further interacts with conserved residues of the NNRT binding pocket. These observations helped to provide a rational explanation of the improved activity of R165481 with WT RT and drug-resistance variants. R221239 is further characterized by a flexible linker to a furan ring at C-6 that makes favorable interactions with Val106, Phe227, and Pro236. It was hypothesized that these interactions also provide a structural basis to rationalize its binding to mutant strains.<sup>25</sup>

<Insert Table 2 here>

#### 4.4. 4-Cycloalkoxy-pyridin-2(1*H*)-one derivatives

As discussed above, the SAR of the C-4 position of the pyridinone ring was largely explored using a number of substituents including arylthio, aryloxy, or benzyl.<sup>22, 24</sup> In 2009, Le Van *et al.* reported a novel series of 4-cycloalkoxy-pyridin-2(1*H*)-one derivatives.<sup>11</sup> The best compounds were **4** and **5**

(Figure 3). These two compounds showed high potency with WT HIV-1 RT and a series of single and double mutants. In addition, both molecules showed low toxicity. The antiviral activity profile is summarized in Table 1. Molecular docking of the newly synthesized pyridinones with a co-crystal structure of RT bound to TNK651 (PDB ID 1RT2, Table 2) showed an expected binding mode characterized by the following interactions: the lactam NH group forms a hydrogen bond with Lys101 and the C-4 group is located in the hydrophobic pocket interacting with Tyr181, Tyr188, Phe227, and Trp229. In that binding model, the C-5 substituent points towards Try181. The C-3 group is close to Pro236 which is the most flexible region of the allosteric pocket. Docking of compound **4** with WT and K103N mutants suggested that this compound and other structural derivatives active against mutant strains show activity because their ability to adopt a different binding mode with the mutated binding pocket. For example, the carbonyl of the pyridinone ring is close to the amide group of Asn suggesting a favorable electrostatic interaction with the K103N mutant.<sup>11</sup>

#### 4.5. Development of pyridin-2(1H)-one/TNK651 hybrids

In 2013 Li *et al.* published the design of novel compounds<sup>27</sup> based on a hybrid design of the Merck pyridinones (Figure 3) and TNK651 (Figure 2B) previously designed by the same authors (*vide supra*).<sup>20</sup> The new series of pyridin-2(1H)-ones were characterized by a cycloalkoxy group at C-4 with diverse substituents at C-3 and C-6. During the design of the new compounds it was hypothesized that including a saturated ring at C-4 could increase the molecular flexibility of the molecule that is known to be desirable feature to retain potency with drug resistance strains (*vide supra*). It was also hypothesized that including a methyl group at the ring at C-4 could enhance the interactions with the conserved residue W229 and, at the same time, reducing the  $\pi$ - $\pi$  stacking interactions with Y181. The substituents at C-6 were chosen based on the comparison with the structure and SAR information available for TNK651. In particular, it was proposed that a substituent with a relative long side chain at this position could extend into a tunnel that connects the allosteric with the catalytic sites surrounded

by Pro236 and Tyr318. These interactions are known to favor the affinity with WT HIV-1 and clinically relevant mutant strains. The compound with the best activity profile was LAM-*trans* that has an isopropyl group in C-3, a substituted cyclohexyl at C-4 and phenylethyl at the C-6 side chain (Figure 3). This molecule showed an  $IC_{50} = 0.003 \mu\text{M}$  in an enzyme-based assay, an  $EC_{50} = 0.036 \mu\text{M}$ , and cytotoxicity  $CC_{50} = 114.38 \mu\text{M}$  (Table 1). The corresponding selectivity index (SI) was 3177, that was significantly better than the SI for nevirapine under the same assay conditions.<sup>27</sup> Furthermore, LAM-*trans* showed strong inhibition of the single mutations K103N and Tyr181C with  $EC_{50}$  of 0.075  $\mu\text{M}$  and 0.190  $\mu\text{M}$ , respectively. Notably, it was observed that the configuration of the substituted cyclohexyl group at C-4 had a dramatic impact in the antiviral activity, in particular for the mutant strains. This is a good example of an ‘activity cliff’ (in particular a 3D activity cliff) *i.e.*, compounds with similar structure but large difference in the activity profile.<sup>28, 29</sup> The high sensitivity of the activity profile to the configuration of the molecule can be associated with the flexibility of the binding pocket and, overall, to the induced-fit mechanism of the NNRTIs (*vide supra*).

Li *et al.* also reported docking studies of LAM-*trans* and structural analogues *i.e.*, compound with iodine atom at C-3, with a co-crystal structure of RT bound to TNK651 (Table 2). Docking models supported the hypothesis that the phenylethyl at C-6 makes interactions with Tyr318 and Pro236. Docking of the analogue with an iodine atom at C-3 also confirmed the hypothesis of a halogen bond with the carbonyl oxygen of Tyr188. As expected, in the docking models, the 2'-methyl-cyclohexyl substituent at C-4 is oriented towards the hydrophobic sub-pocket formed by the residues Tyr188, Phe227, and Trp229. Compound LAM-*trans* was also docked with crystallographic structures of two mutant forms of RT, K103 and Y181C. In agreement with the experimental data, docking results showed that some of the protein-ligand interactions detected with the native form were lost.<sup>27</sup> However, some key interactions with the binding pocket remained; this explained in part the activity of LAM-*trans* with mutant strains. For example, the hydrogen bond with Lys101 was conserved in the docking model with the Y181C mutant. Also with the Y181C mutant, the interactions of the C-6

phenylethyl group with Tyr318 and Pro236 were preserved. With both mutants, the cyclohexyl ring of LAM-*trans* made interactions with the hydrophobic pocket formed by Tyr188 and Trp229.<sup>27</sup>

Based on the chemical structure of LAM-*trans* (Figure 3) in 2015 Cao *et al.* published the exploration of the SAR modifying positions 3 and 4 of the pyridin-2(1*H*)-one ring keeping constant a 6-phenylethyl at the C-6 side chain.<sup>30</sup> At position C-3 authors evaluated the SAR of halogens I and Br, and two substituents with steric or lipophilic character: isopropyl and the N,N-dimethyl. One of the ideas to including halogens at C-3 was to favor an halogen bond with the carbonyl oxygen of the Tyr181 residue. At position C-4 Cao *et al.* explored the SAR with saturated and aromatic rings with oxygen, sulfur and nitrogen linkers (O, S, NH) that have different electronegativity into the bridging site.<sup>30</sup> Out of 16 synthesized compounds, the isopropyl group at C-3 and phenyl group at C-4 yielded the best HIV-1 RT inhibitory activity in an enzyme-based assay. Compound **6** with an oxygen linker (Figure 3) also showed the best antiviral activity in a cell-based assay with an EC<sub>50</sub> = 0.056 μM, and cytotoxicity CC<sub>50</sub> = 195.2 μM. The SI (3467) was better than the value obtained for nevirapine under the assay conditions of the study. Docking studies of a closely related analogue of **6** but with a sulfur linker with a co-crystal structure of RT bound to TNK651 (Table 2) showed the expected binding conformation characterized by two notable interactions, namely: hydrogen bond interaction of the NH group of the pyridine-2(1*H*)-one ring with the backbone oxygen of Lys101 and π-stacking interactions of the C-4 phenyl group with Tyr188. The docked compound showed a comparable binding mode as the position of the co-crystal ligand. Further docking of a structural analogue of **6** but with iodine atom at C-3 also supported the hypothesis of a halogen bond with the carbonyl oxygen of Tyr188.<sup>30</sup> It remains to test the activity of compound **6** with resistant mutant strains.

#### 4.6. Toward new generations of pyridinones with potential improved activity profile

Inspired by the successful design of pyridin-2(1*H*)-one / HEPT hybrids along with docking studies and structural analysis of the predicted binding modes of the Merck pyridonones with the crystallographic

structures of NNRTIs such as UC-781 (Figure 2B), in 2007 Medina-Franco *et al.* designed *in silico* pyridin-2(1*H*)-one/UC-781 hybrids. A representative molecule proposed in that study was compound 7 in Figure 4A.<sup>31</sup> In contrast to introduce a substituent with an aromatic ring at C-4, in that study was proposed the strategy of including an aliphatic substituent at the same C-4 position. The aliphatic substituent should be able, at least in principle, to make interactions with conserved residues such as Trp229. In addition, instaurations in the aliphatic substituent could still make the  $\pi$ - $\pi$  aromatic interactions with Tyr181 but such interactions should be less significant as compared to the  $\pi$ - $\pi$  aromatic interactions of the parent Merck pyridinones.<sup>19</sup> Based on this approach, Chávez *et al.* designed and synthesized a series of compounds such as DH-6, DH-7, DH-10, DH-11, and DH-13 (Figure 4A).<sup>32</sup> These compounds are characterized by having an aliphatic substituent at C-4 position and different polar groups at C-3.

<Insert Figure 4 here>

More recently Chávez *et al.* designed a series of quinolone/UC-781 hybrids. A representative compound is DA-3 in Figure 4B. Similar to the pyridin-2(1*H*)-one/UC-781 hybrids, it is proposed that an unsaturated substituent in C-4 position and a polar group at C-3 could be effective against resistant strains. The synthesis of several pyridin-2(1*H*)-one/UC-781 hybrids has been completed.<sup>33, 34</sup> It remains to test the cytotoxicity and antiviral activity of the newly designed compounds with WT and resistant strains available in the clinic. These biological assays are in progress and are expected to be released in due course.

#### 4.7. Contributions of computational methods to develop pyridin-2(1*H*)-one derivatives

Despite the fact structure-based *de novo* design of different scaffolds of NNRTIs is challenging (due to the induced fit mechanism of these compounds),<sup>6</sup> molecular modeling and computational analysis have played a key role in the multidisciplinary efforts to develop NNRTs, for instance, to develop marketed drugs such as etravirine, and rilpivirine.<sup>9</sup> In light of these successful applications, diverse

computational strategies continue to be part of multidisciplinary teams to design anti-HIV compounds. For example, Tarasova *et al.* recently reported QSAR studies of two large databases, Thomson Reuters Integrity and ChEMBL, with activity data for HIV-1 RT inhibition. The purpose of that work was focused on method development in regards to assess the feasibility of using these large databases for QSAR modeling.<sup>35</sup> In a separate but also recent work, Kurczyk *et al.* published a ligand-based virtual screening of 1.5 million compounds commercially available to identify novel chemotypes with anti-HIV-1 activity. The first steps of the virtual screening were focused on inhibitors of HIV integrase. Authors of that work identified two novel chemotypes with promising antiretroviral activity and good candidates to initiate a hit optimization program.<sup>36</sup> A third recent example is the *in silico de novo* design of novel NNRTIs using a bio-molecular modelling approach.<sup>37</sup> Computational methods have helped to advance the development of pyridin-2(1*H*)-one derivatives. As discussed above, computational studies have contributed to provide hypothesis that explain the activity of Merck pyridinones and newly designed compounds against WT and mutant strains. Most of the studies involve molecular docking, using various software programs, with crystallographic structure of both wild-type and mutant strains. Table 3 summarizes representative computational studies including the major outcomes.

<Table 3 here>

In addition to molecular docking, other methods have also contributed to understand the SAR of data sets of compounds in a retrospective or prospective manner. For instance, in 2004 Medina-Franco *et al.* reported three-dimensional quantitative SAR analyses (3D-QSAR) of 40 Merck pyridinones.<sup>38</sup> In that study, molecular docking was used as a strategy to align the structures for Comparative Molecular Field –CoMFA- and Comparative Molecular Similarity Analysis -CoMSIA-. The 3D-QSAR models, in combination with docking, supported the hypothesis that the interaction between the benzoxazole ring of Merck pyridinones with Tyr181 play an important role to stabilize the complex. Therefore, the

outcome of that study provided insights to design novel pyridin-2(1*H*)-one derivatives with potential activity against mutant strains.<sup>38</sup>

A year later, in 2005 it was published a 2D-QSAR study for more than 40 Merck pyridinones using a *k* nearest neighbor approach.<sup>39</sup> The most promising QSAR models were employed to screen the US National Cancer Institute database. Derivatives of the pyrazolo[3,4-*d*]pyrimidine (such as NSC11635) and phenothiazine (NSC 127) type were identified as putative NNRTIs leads. Hit molecules were subject of molecular docking to explore the putative binding mode. Docking results supported the potential activity of the hit molecules.<sup>39</sup>

In 2006 Bajaj *et al.* developed a novel descriptor called *augmented eccentric connectivity index* to develop 2D-QSAR models for a data set of 72 3-aminopyridin-2(1*H*)-ones. Authors concluded that the novel descriptor performed better than other previously used descriptors. A total of 80.6 % of the compounds on that data set were correctly predicted by the model.<sup>40</sup>

More recently, in 2013 Debnath *et al.* published a comprehensive 3D-QSAR (specifically, CoMFA and CoMSIA) study of 178 4-benzyl/benzoyl pyridin-2(1*H*)-ones using HIV-1 RT activity from WT and three mutants: K103, Y181C, and Y188L.<sup>41</sup> For this studies the molecular alignment (*i.e.*, possible bioactive conformations) were taken from crystallographic structures and molecular docking. From the CoMFA and CoMSIA models was concluded that the C-3, C-4 and C-6 positions of the pyridionone ring were very important for a broad-spectrum anti-HIV-1 RT activity. It was also concluded that an electronegative field between the pyridinone and aryl moieties is a common requirement for the activities.<sup>41</sup> Based on the 3D-QSAR and docking studies, authors designed compounds with putative activity against WT and mutant strains K103N, Y181C, and Y188L. One example is molecule **VC1** (Figure 4).

## 5. CONCLUSIONS

Pyridine-2(1*H*)-ones represent a major structural class under development as NNRTIs. Structure-based analysis, QSAR and docking studies, combined with medicinal chemistry approaches and biological screening have played a key role in the multidisciplinary effort to develop advance generations of pyridine-2(1*H*)-ones derivatives as a promising class of NNRTIs. Crystallographic structures of pyridine-2(1*H*)-ones are guiding the structure-based design of novel sub-classes with promising activity profile against mutant strains. An effective strategy to improve the activity profile of pyridinone derivatives has been the preparation of hybrids with other NNRTIs. Novel generations of compounds are being designed based on: 1) the accumulated knowledge of the SAR of pyridine-2(1*H*)-ones; 2) the known requirements of ligand flexibility to adapt to the mutated binding pocket; and 3) the desirable feature to make favorable interactions with conserved residues. Amongst the promising novel classes of compounds are pyridin-2(1*H*)-one/UC-781 and quinolone/UC-781 hybrids. These new classes may enter into clinical development for the treatment of AIDS.

## ACKNOWLEDGMENTS

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## TABLES

**Table 1.** Activity profile of selected pyridinone derivatives and reference NNRTIs from other chemical classes against wild-type and mutant HIV-1 RTs

Compound	IC <sub>50</sub> RT ( $\mu$ M)	EC <sub>50</sub> ( $\mu$ M) WT RT LAI cell line	EC <sub>50</sub> ( $\mu$ M) K103N	EC <sub>50</sub> ( $\mu$ M) Y181C	EC <sub>50</sub> ( $\mu$ M) K103N/Y181C	EC <sub>50</sub> ( $\mu$ M) L100I/K103N	SI <sup>a</sup>	Ref
<b>2</b>	0.02	0.0002 LAI cell line	NA	NA	NA	NA	>5000	22
<b>3</b>	NA	0.0013 LAI cell line	0.003	0.020	0.040	0.020	9000	26
<b>4</b>	NA	<0.001	0.005 (TZM-bl Cells)	0.015 (TZM-bl Cells)	0.107 (TZM-bl Cells)	0.081 (TZM-bl Cells)	>67000	11
<b>5</b>	NA	<0.001	0.002 (TZM-bl Cells)	0.006 (TZM-bl Cells)	0.105 (TZM-bl Cells)	0.044 (TZM-bl Cells)	>82000	11
<b>6</b>	0.089	0.0563	NA	NA	NA	NA	3467	30
R157208	NA	0.001 LAI cell line	0.079	0.794	10	0.794	10000	24
R165481	NA	0.0008	0.001	0.004	0.004	0.008	NA	25
R221239	NA	0.002	0.001	0.005	0.005	0.032	NA	25
LAM- <i>trans</i>	0.003	0.036	0.075	0.190	NA	NA	3177	27
Nevirapine	4.65 <sup>b</sup>	0.190 <sup>b</sup>	>10 <sup>c</sup>	>10 <sup>c</sup>			1074 <sup>b</sup>	
Efavirenz <sup>d</sup>		0.001	0.039	0.002	0.04	>10		
Etravirine <sup>d</sup>		0.002	0.001	0.006	0.005	0.01		
Rilpivirine <sup>d</sup>		0.0004	0.0003	0.0001	0.0008	0.008		

<sup>a</sup> Selectivity Index: ratio of cytotoxicity (CC<sub>50</sub>) to EC<sub>50</sub><sup>b</sup> HIV-1<sub>SF33</sub> infection in TZM-bl cell lines taken from <sup>30</sup><sup>c</sup> Taken from <sup>27</sup><sup>d</sup> EC<sub>50</sub> values in  $\mu$ M taken from <sup>12</sup>

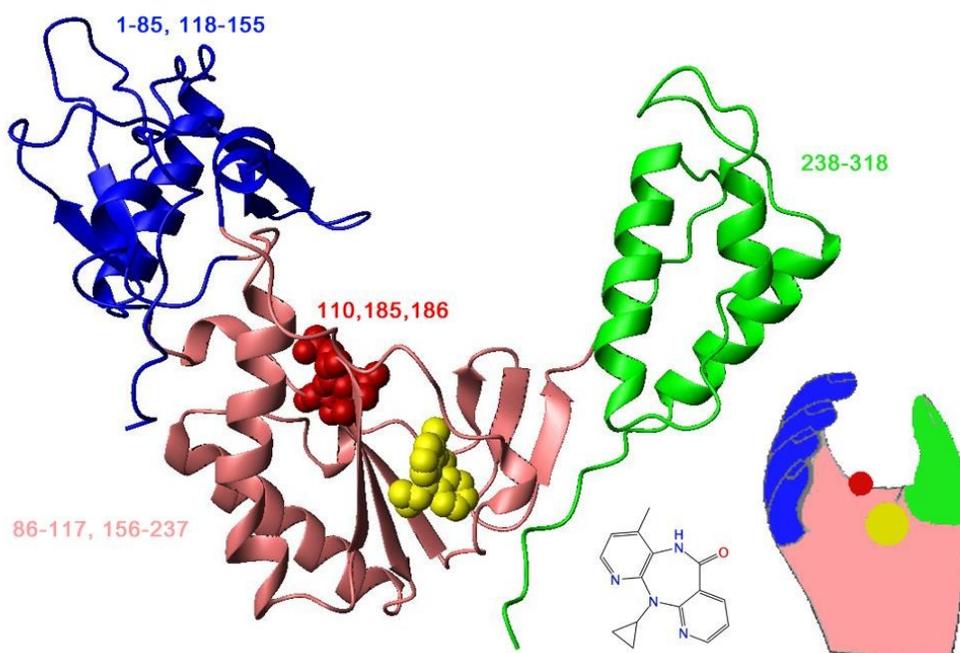
**Table 2.** Crystallographic structures of HIV-1 RT co-crystallized with pyridinone derivatives and other representative NNRTIs discussed in the manuscript

PBD ID	Description	Resolution (Å)	Co-crystallized NNRTI	Reference
2BAN	Wild-type HIV-1 RT/ATP	2.95	R157208	25
2B5J	Wild-type HIV-1 RT	2.90	R165481	25
2BE2	Wild-type HIV-1 RT	2.43	R221239	25
1RT2	Wild-type HIV-1 RT	2.55	TNK651	20
1FKP	K103N mutant in complex with Nevirapine	2.9	Nevirapine	42
1JLA	Y181C mutant complex with TNK-651	2.5	TNK651	43

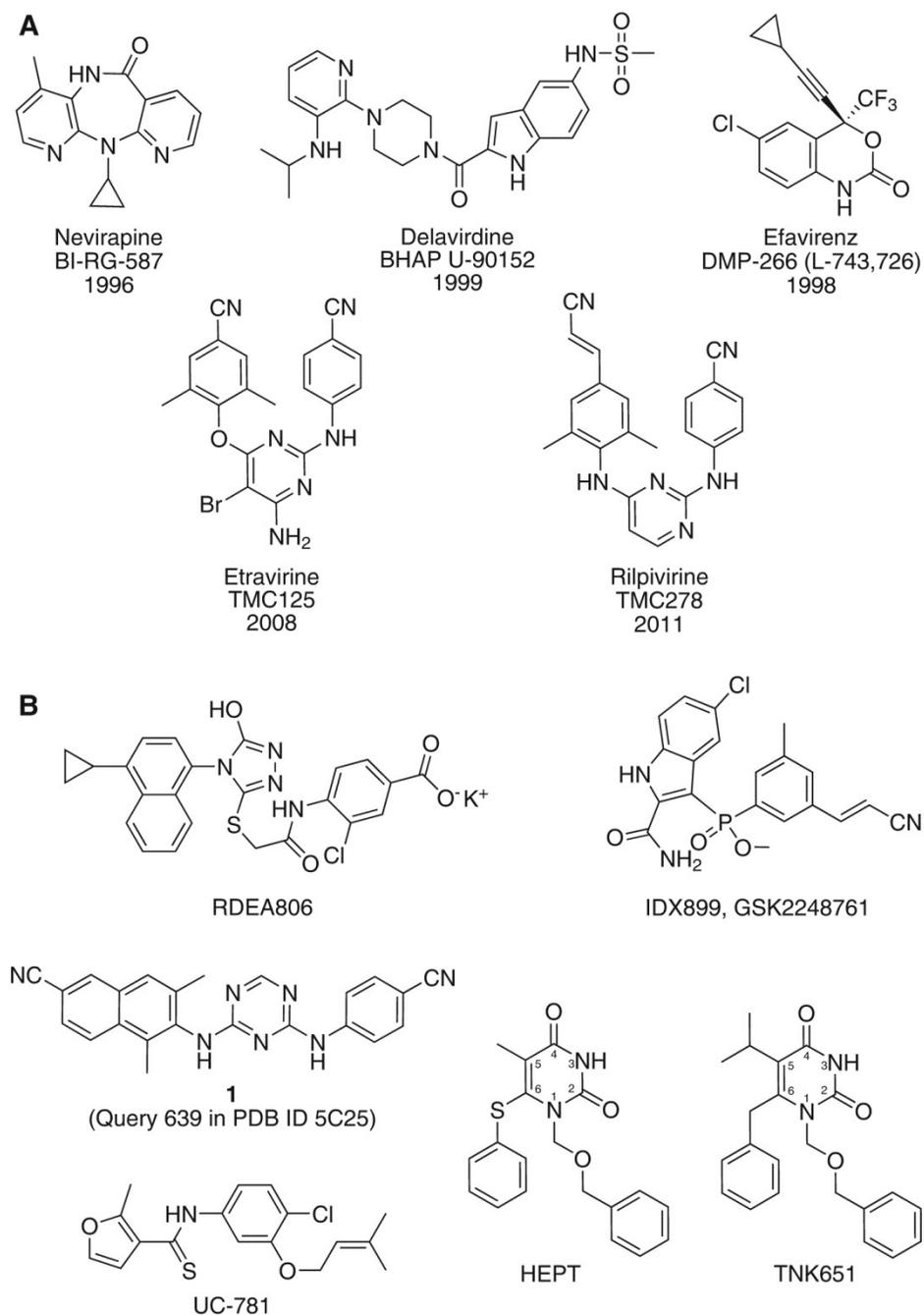
**Table 3.** Representative computational studies towards the development of pyridinone derivatives

Study	Computational approaches	Major outcomes	Ref
Flexible docking of selected Merck pyridinones	Docking with Autodock using crystallographic structures of RT bound to NNRTIs different from pyridinones.	The pyridinones adopt a butterfly-like conformation in the NNRTI binding pocket; the lactam NH group forms the signature hydrogen bond with Lys101; the benzoxazol ring of L-697,661 and L-696,229 make aromatic interactions with Tyr181.	19
Docking and QSAR of 40 Merck pyridinones	3D-QSAR, CoMFA and CoMSIA and docking with Autodock.	Hypothesis: the benzoxazole ring of Merck pyridinones is implicated in the loss of activity upon mutation of Y181.	38
QSAR of 40 Merck pyridinones and virtual screening	2D-QSAR, <i>k</i> Nearest Neighbor and docking with Autodock.	Derivatives of the pyrazolo[3,4-d]pyrimidine and phenothiazine type were identified as putative NNRTIs leads.	39
QSAR of 72 3-aminopyridin-2(1 <i>H</i> )-ones	2D-QSAR using a novel augmented eccentric connectivity index.	80.6 % of the pyridin-2(1 <i>H</i> )-ones of the data set were correctly predicted.	40
Docking and QSAR of 178 4-benzyl/benzoyl pyridin-2-ones	3D-QSAR, CoMFA and CoMSIA and docking with Autodock using PDB IDs 2BAN (WT), 3MED (K103N), 1JKH (Y181C), 2YNF (Y188L).	The C-3, C-4 and C-6 positions of the pyridinone ring were very important for a broad-spectrum anti-HIV-1 RT activity. An electronegative field between the pyridinone and aryl moieties is a common requirement for the activities. Virtual compounds were designed.	41
Docking of <b>4</b> and structural analogues	Docking with GOLD using PDB ID 1RT2 (WT), and K103N mutant (no PDB ID disclosed). Further energy minimization of the complex.	Expected binding orientation: the lactam NH group forms a hydrogen bond with Lys101; the C-4 group is located in the hydrophobic pocket (Tyr181, Tyr188, Phe227, and Trp229). The C-3 group is close to Pro236. <b>4</b> can adopt alternative binding modes with WT (i.e., C-3 or C-6 close to Pro236). In K103N, the carbonyl of the pyridinone ring can make favorable interactions with the amide group of Asn.	11
Docking of LAM- <i>trans</i> and structural analogues	Docking with Autodock Vina using PDB IDs: 1RT2 (WT), 1FKP (resistant K103N mutant), and 1JLA (resistant Y181C mutant).	Hypothesis: the phenylethyl at C-6 makes interactions with Tyr318 and Pro236. Docking models with mutant strains showed a less number of interactions with the binding pocket but some key interactions remained unchanged.	27
Docking of two compounds of the 6-phenylethyl pyridinone family	Docking with GOLD using PDB ID 1RT2 (WT).	Expected binding conformation, similar to the co-crystal ligand TNK651 (hydrogen bond interaction with Lys101 and $\pi$ -stacking with Tyr188). Docking of an analogue with iodine atom at C-3 supported the hypothesis of a halogen bond with the carbonyl oxygen of Tyr188.	30

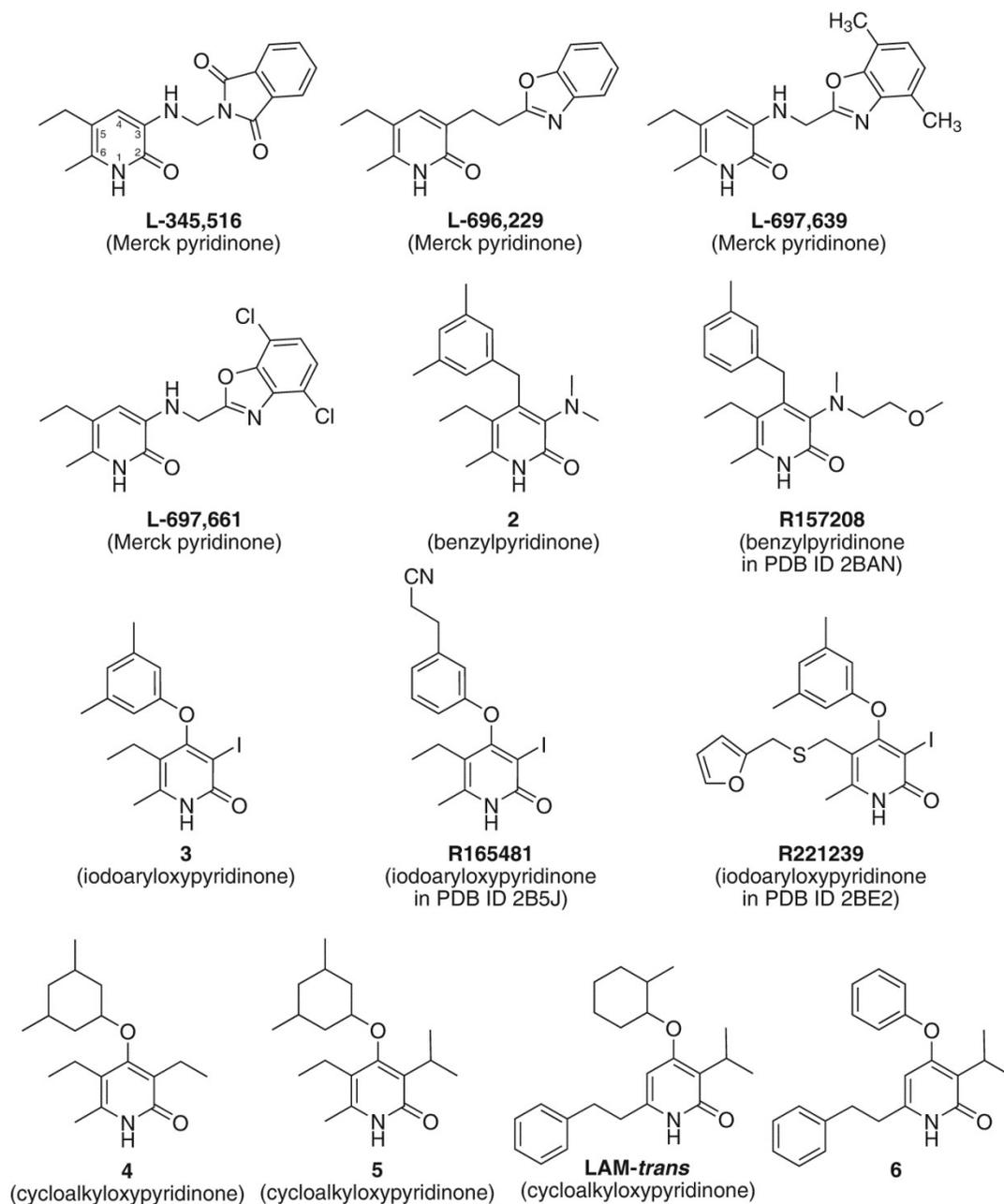
## FIGURES



**Figure 1.** Schematic representation of the three-dimensional structure of HIV-1 reverse transcriptase (RT). This ribbon representation of the RT active domain illustrates its hand-like structure, showing fingers (blue), palm (pink) and thumb (green). The fingers correspond to amino acids 1-85 and 118-155; the palm is associated with amino acid residues 86-117 and 156-237; and the thumb with amino acid residues 238-318. The active site (Asp110, Asp185, and Asp186), where DNA is elongated, is in the palm region. It is also the position of the RT inhibitor nevirapine (yellow) in the non-nucleoside binding pocket. Adapted from [http://www.psc.edu/science/Madrid/getting\\_a\\_grip\\_on\\_aids.html](http://www.psc.edu/science/Madrid/getting_a_grip_on_aids.html)

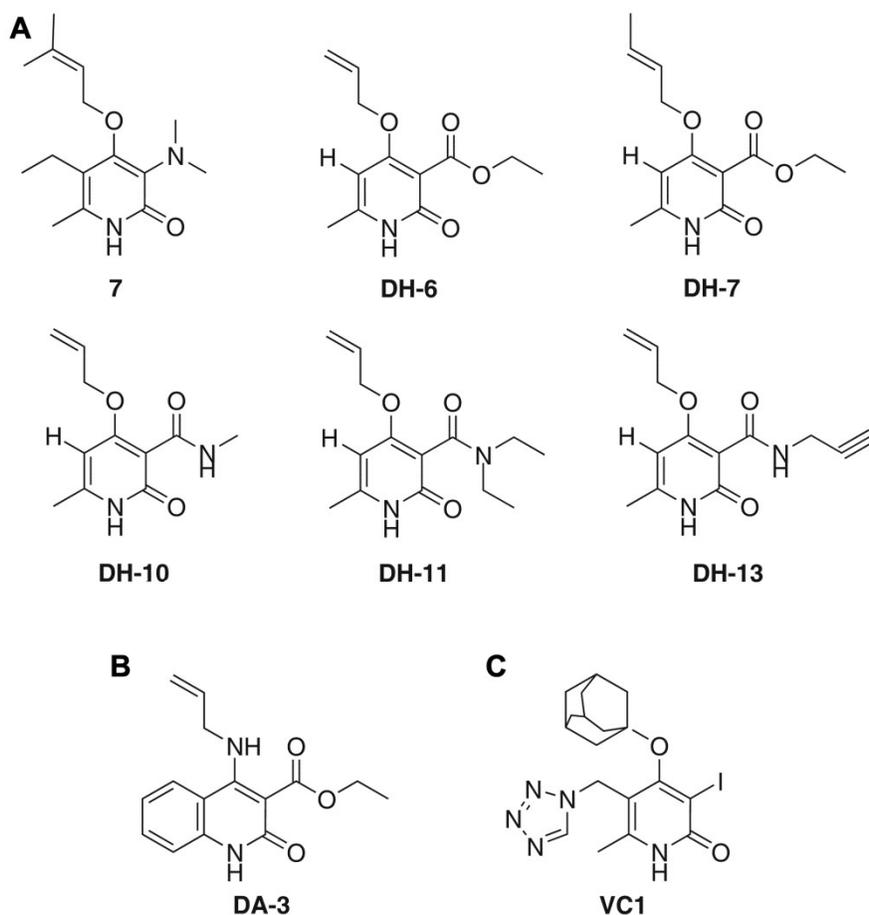


**Figure 2.** Chemical structures of non-nucleoside HIV-1 reverse transcriptase inhibitors: **A)** Approved for clinical use (including year of approval); **B)** Examples of other reference inhibitors discussed in the manuscript including compounds under development.



**Figure 3.** Chemical structures of representative pyridin-2(1*H*)-one derivatives discussed in this work.

The numbering of the pyridinone ring is shown in structure L-345,516. The activity profile of selected compounds against wild-type and mutant strains is summarized in Table 1.



**Figure 4.** Chemical structures of representative newly designed pyridine-2(1*H*)-ones and related compounds with potential activity against wild-type and mutant strains. **A)** Pyridin-2(1*H*)-one/UC-781 hybrids designed by a structure-based approach. **B)** Quinolone/UC-781 hybrid designed by a structure-based approach. **C)** 3-iodo-pyridin-2(1*H*)-one designed by 3D-QSAR and docking. See text for details.

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Multidisciplinary research involving medicinal chemistry, computational design and biological screening have advanced pyridin-2(1*H*)-one derivatives as a promising class of non-nucleoside reverse transcriptase inhibitors for the treatment of HIV/AIDS.

