# Contemporary medicinal chemistry strategies for the discovery and development of novel HIV-1 nonnucleoside reverse transcriptase inhibitors

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## ABSTRACT

Currently, HIV-1 nonnucleoside reverse transcriptase inhibitors (NNRTIs) are a major component of the highly active antiretroviral therapy (HAART) regimen. However, the occurrence of drug-resistant strains and adverse reactions after long-term usage have inevitably compromised the clinical application of NNRTIs. Therefore, the development of novel inhibitors with distinct anti-resistance profiles and better pharmacological properties is still an enormous challenge. Herein, we summarize state-of-the-art medicinal chemistry strategies for the discovery of potent NNRTIs, such as structure-based design strategies, contemporary computer-aided drug design, covalent-binding strategies, and the application of multitarget-directed ligands. The strategies described here will facilitate the identification of promising HIV-1 NNRTIs.

# 1. INTRODUCTION

Acquired immune deficiency syndrome (AIDS) caused by human immunodeficiency virus-1 (HIV-1) still remains a serious epidemic disease that threatens human health worldwide.[1] According to the latest statistical data from the Joint United Nations Programme on HIV/AIDS (UNAIDS), there were 37.7 million HIV-infected patients globally and 0.68 million deaths from HIV-related illnesses in 2020.[2] In the fight against AIDS, a combination of multiple antiviral agents, namely, the highly active antiretroviral therapy (HAART) regimen, as the standard therapy for HIV, has successfully controlled the spread of AIDS and reduced the high-mortality disease into a chronic disease.[3] The HAART regimen, which typically involves well-known reverse transcriptase (RT) inhibitors, has proven to be the most effective and safe treatment to suppress viral replication and reduce viral spread.[4]

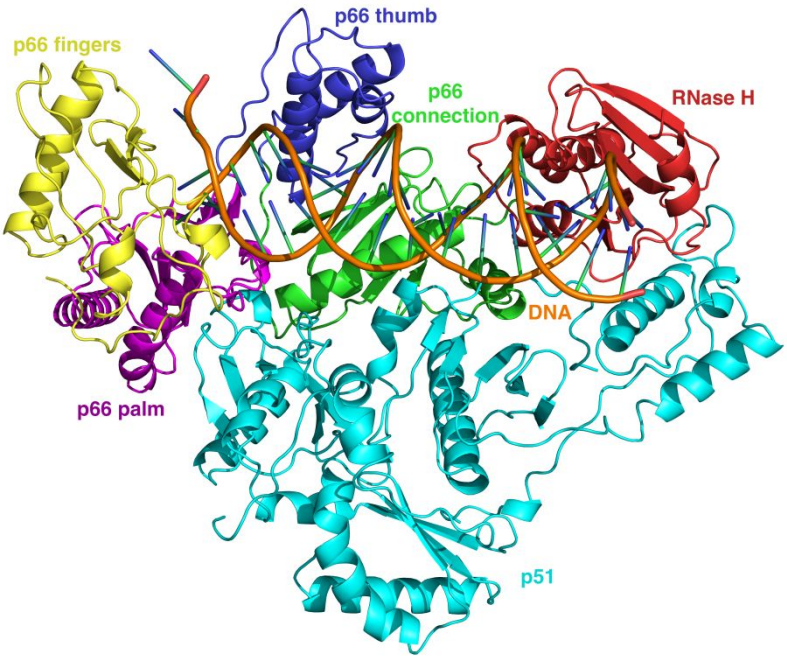


**Figure 1.** The X-ray crystal structure of HIV-1 RT (PDB code: 1RTD). The image was generated by PyMOL software.

Among all the therapeutic targets related to the HIV-1 infectious disease, RT is an attractive target because of its well-solved X-ray crystallographic structure and high tolerance to diverse chemical molecules.[5, 6] In the heterodimeric protein RT, the p51 subunit does not have a catalytic function, but only participates in conformational regulation for the p66 subunit.[7] The p66 subunit, which responsible for performing all catalytic activities, consists of the polymerase domain and the RNase H domain. The shape of the polymerase domain is similar to that of the right hand, so the four constituent subdomains are referred to as fingers, palm, thumb, and connection. The RNase H domain is associated with the polymerase domain through the connection subdomain (**Figure 1**).[8] The RT enzyme plays a critical role during viral replication and is responsible for the reverse transcription process.[9, 10] To replicate inside the host cell, RT first exhibits RNA-dependent DNA polymerase activity and uses viral RNA as the template to synthesize RNA:DNA double strands. Subsequently, the RNase H domain can degrade the RNA strand of hybrid intermediates. Finally, RT serves as a DNA-dependent DNA polymerase to transform the remaining single-stranded DNA into the double-stranded DNA product.[11]

RT inhibitors are generally classified into nucleoside reverse transcriptase inhibitors (NRTIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs) owing to different binding sites and chemical classes of compounds.[12] The competitive inhibitor NRTIs are converted intracellularly into their corresponding triphosphate forms, with a structure similar to natural nucleotides, through three different phosphorylation cascades and are then connected into growing DNA strands by HIV-1

RT. These nucleotide analogs compete with their natural counterparts and prevent insertion of the next endogenous nucleotide. NNRTIs can disrupt the normal function of RT by occupying the NNRTI-binding pocket (NNIBP), thereby affecting the conformation of the active catalytic site.[13, 14] Unlike NRTIs, NNRTIs do not compete for natural substrates and therefore will not directly interfere with DNA polymerization but instead block the reverse transcription process by inducing conformational changes of RT upon binding to NNIBP as allosteric inhibitors.[15]

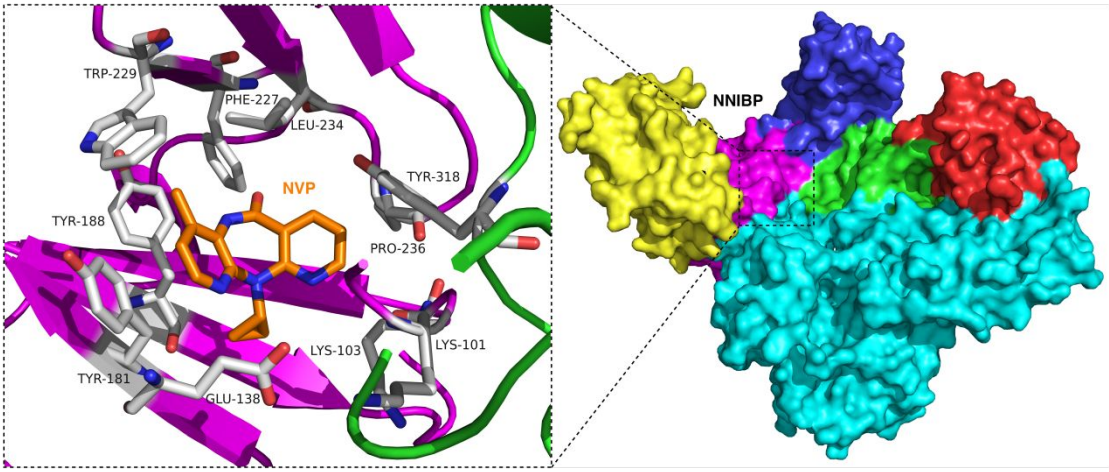


**Figure 2.** The binding modes of NVP (orange) within the NNRTI-binding pocket (PDB code: 1VRT). The image was generated by PyMOL software.

Because of the rapid development of resistance to a single drug, NNRTIs have been recognized as essential ingredients in the HAART regimen.[16] The single-tablet combination regimen shows obvious advantages, including lower doses and higher genetic barriers to resistance due to synergistic inhibition between multiple drugs, as well as improved patient compliance.[17] The integrase inhibitor dolutegravir in combination with NNRTI rilpivirine (Juluca®) represents the first approved two-drug single-tablet regimen for maintenance therapy.[18] According to the data of two phase III

non-inferiority trials, the dolutegravir+rilpivirine coformulation was shown to be non-inferior to the current therapy for the maintenance of HIV-1 suppression in treatment-experienced individuals. Moreover, this well-tolerated single-tablet regimen was characterized by safe cardiovascular, renal, bone, and lipid toxicity profiles.[19, 20] In the current landscape of antiretroviral options, there remains an urgent need for novel classes of NNRTIs with favorable tolerance and convenience to make potential NNRTI-based regimens more attractive.

To date, six NNRTI drugs have been approved by the U.S. FDA: including three first-generation inhibitors nevirapine (**1**, NVP), delavirdine (**2**, DLV), and efavirenz (**3**, EFV); two second-generation inhibitors etravirine (**4**, ETV) and rilpivirine (**5**, RPV); and the newly approved drug doravirine (**6**, DOR) (**Figure 3**).[21, 22] However, the occurrence of resistance has compromised the clinical use of first-generation drugs, especially the two most prevalent single mutants, K103N and Y181C, selected by nevirapine and efavirenz. Although the second-generation NNRTIs belonging to the diarylpyrimidine (DAPY) family exhibit prominent antiviral activity toward most of the clinically common mutations, they suffer from poor aqueous solubility and unfavorable pharmacokinetic properties. Compared to both DAPY-type drugs ETV and RPV, the pyridone derivative DOR displayed more potent antiviral activity against the two most prevalent mutations K103N and Y181C, while inferior potency was observed in terms of mutations V106A, F227L, and L234I in clinical trials.[23] Therefore, the discovery of novel NNRTIs with distinct resistance profiles and better pharmacological properties remains an imminent and challenging toned in the research community.

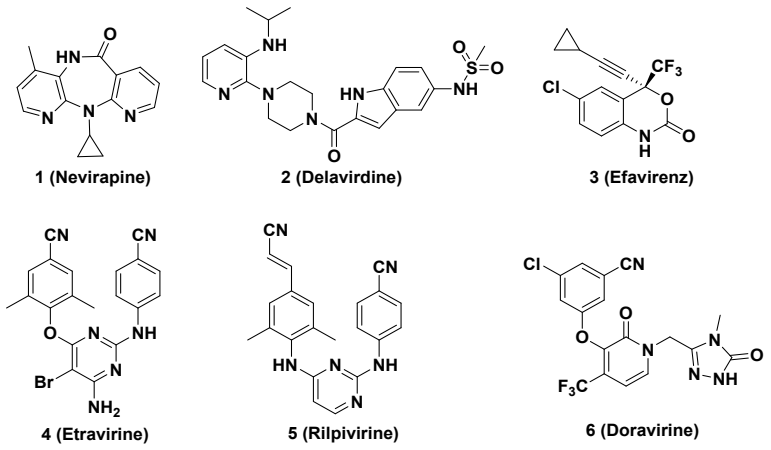


**Figure 3.** Six NNRTI drugs approved by the U.S. FDA.

The RT allosteric binding site is not directly related to the function of the catalytic active site of the polymerase domain, so NNIBP does not need to be highly conserved and can adapt to mutations without significantly impairing normal enzyme function or affecting viral fitness.[24] Almost all residues in NNIBP can be relatively easily mutated, and most of the resulting mutations can confer resistance to NNRTIs.[25, 26] Therefore, resistance-associated mutations reduce susceptibility by altering the interaction between inhibitors and NNIBP *via* multiple mechanisms, such as steric hindrance (L100I and G190A/S), the prevention of effective entry into the pocket (K101E/P, K103N, and E138K), alteration of the hydrophobic interactions (V106A, V179D, and F227C/L), and loss of π–π stacking interactions with aromatic residues (Y181C and Y188L). Specifically, L100I and G190A/S mutate to a bulky amino acid, so the increase in the volume of the amino acid causes steric hindrance between the ligand and the protein by altering the shape of NNIBP.[27] Amino acid mutations at the K101, K103, and E138 positions in the entrance channel can interfere with the entry of NNRTI by extending the side chain out of the pocket.[28] Mutations occurring in hydrophobic residues V106, V179, and F227 confer high levels of resistance by weakening the

hydrophobic interaction with NNRTIs.[29] Similarly, the disappearance of crucial π–π stacking interactions with the aromatic amino acids Tyr181 and Tyr188 causes significantly reduced susceptibility to Y181C and Y188L.[30, 31]

Herein, we will elaborate on the discovery and optimization of NNRTIs over the last two decades. The following contents comprise not only traditional medicinal chemistry principles but also, in certain chapters, the elucidation of nonclassical antiviral strategies to block viral infection, including modern computer-aided drug design and targeted covalent inhibitors. In addition, the inspiration and guidance of these previous research efforts are discussed in detail. Most importantly, our current viewpoints of the challenges and prospects of future drug development work are also addressed. We hope that this review will contribute to highlighting the significant role and potential of diverse medicinal chemistry strategies in the evolution of the ongoing search for new and efficient NNRTIs.

## 2. DISCOVERY OF HIV-1 NNRTIS LEADS

The discovery of lead compounds represents one of the most significant research areas in medicinal chemistry and usually relies on screening existing compounds to find drug-like molecules that act on a specific target.[32] Although traditional in-house chemical library screening to identify leads through biochemical assays is conceptually more sophisticated, the approach suffers from a heavy workload and false positives. In contrast, current technologies, including virtual screening and diversity-oriented synthesis combined with rapid screening as complementary strategies, have fully

ensured the efficient progress of novel NNRTI discovery.[33, 34]

## 2.1. Virtual Screening and Structure-Based Design Strategy

The cost-intensive characteristics of the traditional drug development process, coupled with the drug resistance challenge conferred by the easy mutation of viral genes, highlight the importance and urgency of the rapid discovery of hit compounds. In the past few decades, the application of computational technologies such as virtual screening has accelerated lead identification and rationalized further structural optimization, thereby making the drug design process more goal-oriented and economical.[35-37]

By virtually screening the commercially available *ZINC* compound library based on three different conformations of RT structures, compound **7**, with an $EC_{50}$ value of 4.8 μM toward the wild-type (WT) strain, was identified as a promising hit for further optimization.[38] Subsequently, optimization of **7** was carried out based on free energy perturbation (FEP) calculations of NNRTI/RT complexes, which was applied to determine the optimal substitution patterns of the benzene moiety and evaluate the feasibility of replacing the methylene linker with oxygen. Catechol diether **8**, featuring terminal uracil and cyanovinylphenyl moieties, exhibited extraordinarily robust potency against the WT strain ($EC_{50}$ = 55 pM).[39] Cocrystal structures of RT and **8** suggested that the cyanovinyl group contacts Trp229 closely and accommodates it in the pocket extremely well (**Figure 4**). In addition, more significant contacts with Lys103 *via* hydrogen bonds were observed in the binding mode. In particular, the chlorine atom forms a halogen bond with Pro95 to greatly improve the potency.[40]

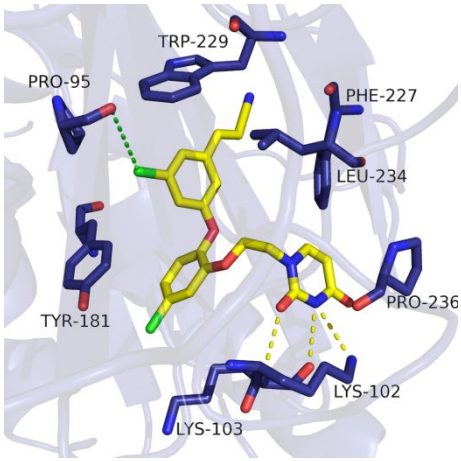


**Figure 4.** Cocrystal structures of compound **8** (yellow) and WT RT (PDB code: 4H4M). Hydrogen bonds are shown as yellow dotted lines, and the halogen bond is indicated with a green dashed line.

Although **8** exhibited picomolar inhibitory activity toward the WT strain, significantly decreased activities were observed for the Y181C and K103N/Y181C mutants. Therefore, based on the predictions of the computational method, eliminating the chlorine atom of the central scaffold afforded analog **9** with improved resistance profiles and solubility. Analysis of cocrystal structures indicated that the reduced steric hindrance caused by the absence of 5-Cl allows compound **9** to adopt favorable binding modes in all three complexes and maintain key hydrogen-bonding interactions with surrounding residues (**Figure 5**).[41]

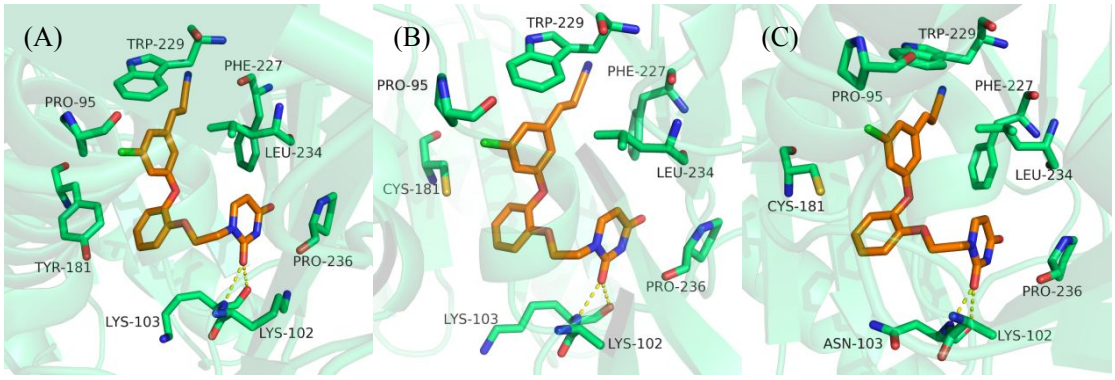


**Figure 5.** (A) Cocrystal structures of compound **9** (orange) and WT RT (PDB code: 4RW8); (B) Cocrystal structures of compound **9** (orange) and Y181C RT (PDB code: 4RW9); (C) Cocrystal

structures of compound **9** (orange) and K103N/Y181C RT (PDB code: 4RW7). Hydrogen bonds are shown as yellow dotted lines.

However, the electrophilic cyanovinyl group in **8** and **9** might act as a Michael acceptor; further modification work was focused on the replacement of the cyanovinylphenyl group, especially considering the possibilities of 6/5 heterobicyclic groups. In accordance with the Monte Carlo/free energy perturbation prediction results, the most potent 2-cyanoindolizinyl derivative, **10**, with suitable aqueous solubility was proven to effectively inhibit the WT and K103N/Y181C strains. Cocrystal structures showed that **10** forms intensive π–π stacking interactions and is involved in ion-dipole interactions or hydrogen bonds (**Figure 6A**).[42] In addition, **10** showed robust potency against the K101P mutant strain (a low-frequency mutation with markedly reduced the susceptibility to both EFV and RPV) with an $EC_{50}$ value of 1 nM. Crystallographic studies revealed that **10** does not rely on salt bridge stabilization or direct backbone hydrogen bonding with Lys101, so the K101P mutation will not damage the binding affinity relative to that of WT RT (**Figure 6B**).[43]

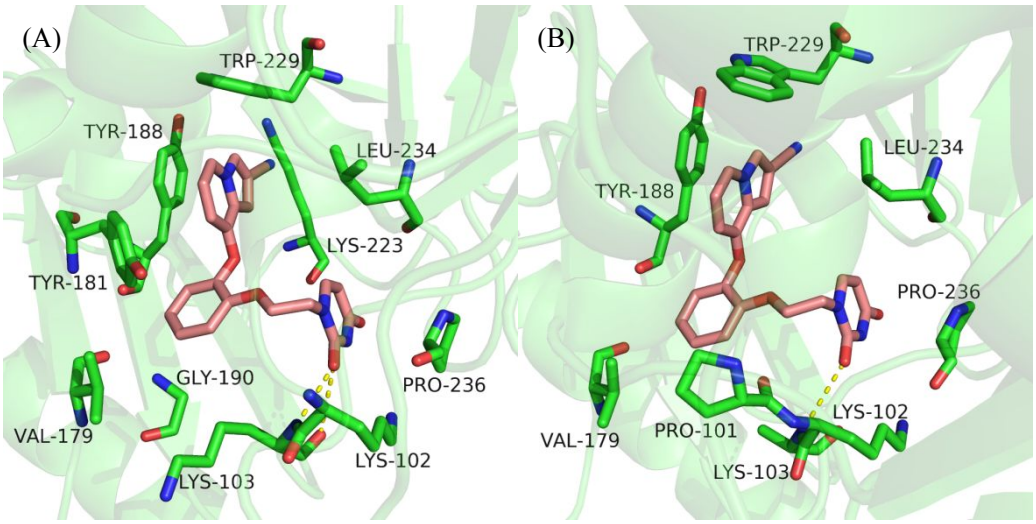


**Figure 6.** (A) Cocrystal structures of compound **10** (pink) and WT RT (PDB code: 4MFB); (B)

Cocrystal structures of compound **10** (pink) and K101P RT (PDB code: 5C42). Hydrogen bonds are shown as yellow dotted lines.

In general, the antiviral activities of NNRTIs almost always decrease significantly for a double variant compared to a single variant. However, the potency of **10** against the Y181C mutant was approximately 30-fold less than that of the K103N/Y181C mutant. To improve the activity of catechol diethers toward the Y181C mutant, the introduction of a bulky naphthalene ring under the guidance of the BOMB program was expected to establish additional π–π interactions. The resulting compounds, 2-naphthyl analog **11** and 1-naphthyl analog **12**, possessed markedly improved anti-resistance profiles compared to **10** in the case of the Y181C mutation (**Figure 7**).[44, 45]

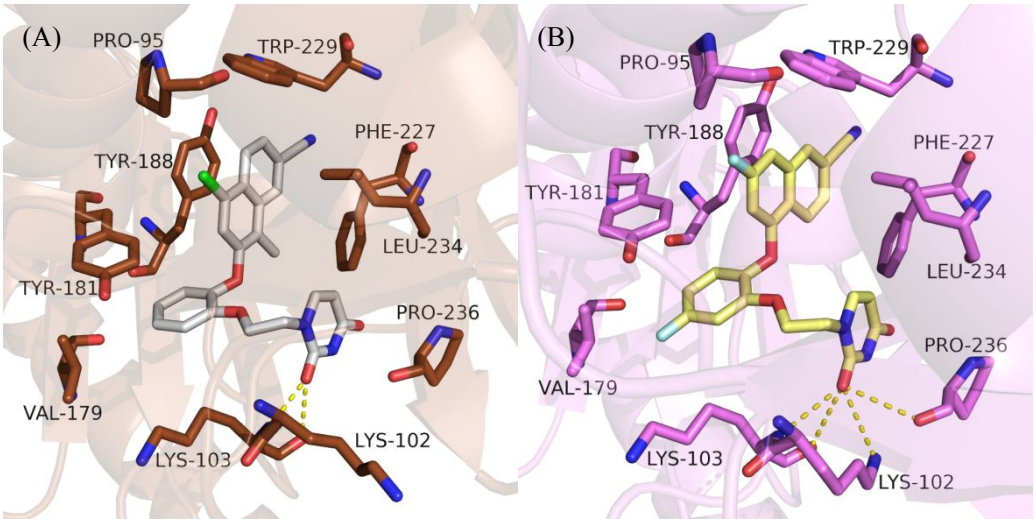


**Figure 7.** (A) Cocrystal structures of compound **11** (white) and WT RT (PDB code: 5TER). (B) Cocrystal structures of compound **12** (yellow) and WT RT (PDB code: 5TW3). Hydrogen bonds are shown as yellow dotted lines.

Further preclinical studies proved the significance of **12** with not only marked activity toward WT and mutant strains but also with desirable *in vivo* pharmacokinetic properties, no substantial cytotoxicity, and off-target effects (**Table 1**). Moreover, the

development of a long-acting nanoformulation of **12** allowed the plasma drug concentration and antiviral efficacy to be continuously maintained for nearly three weeks after a single-dose administration (**Figure 8**).[46] Considering the efficacy of **12** in HIV-1-infected humanized mice and synergistic antiretroviral potency with existing anti-AIDS drugs, it will likely become a promising candidate for preexposure prophylaxis (PrEP).

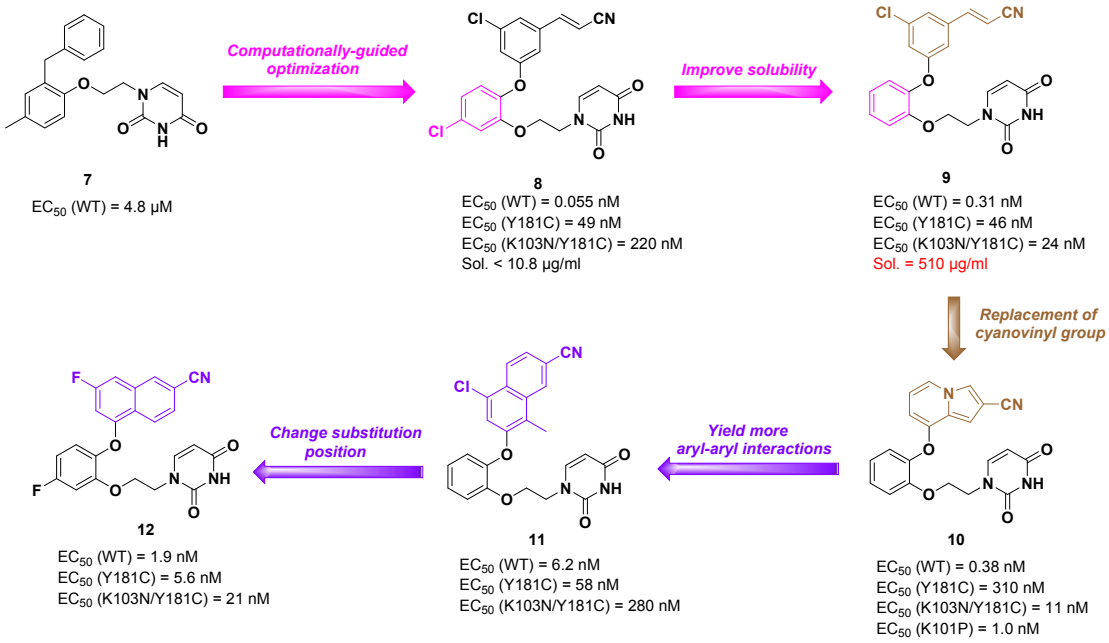


**Figure 8.** Discovery and optimization of a new class of catechol diether-based NNRTIs.

**Table 1.** Antiviral Activity and Cytotoxicity of Compounds **8**−**12**.

| Compd | $EC_{50}$ (nM)[a] | | | $CC_{50}$ (μM)[b] |
|---|---|---|---|---|
| | WT | Y181C | K103N/Y181C | |
| **8** | 0.055 | 49 | 220 | 10 |
| **9** | 0.31 | 46 | 24 | 18 |
| **10** | 0.38 | 310 | 11 | >100 |
| **11** | 6.2 | 58 | 280 | >100 |
| **12** | 1.9 | 5.6 | 21 | >100 |
| **NVP**[42] | 110 | >100000 | >100000 | >100 |

| | | | | |
|---|---|---|---|---|
| **EFV**[42] | 2 | 10 | 30 | 15 |
| **ETV**[42] | 1 | 8 | 5 | 11 |
| **RPV**[42] | 0.67 | 0.65 | 2 | 8 |

[a]$EC_{50}$: The $EC_{50}$ value is defined as the dose required to achieve 50% protection of the infected MT-2 cells by the MTT colorimetric method; antiviral curves were established from triplicate samples at each concentration.

[b]$CC_{50}$: The $CC_{50}$ value is defined as the dose required to achieve 50% inhibition of MT-2 cell growth by the MTT colorimetric method; the toxicity curves were established from triplicate samples.

## 2.2. CuAAC Click Chemistry-Based Combinatorial Libraries

Rapid synthesis and direct screening of combinatorial compound libraries through copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) click chemistry proved to be an important approach for exploring SARs and identifying hit compounds.[47-51] As an extension of the click reaction concept, this method has the advantages of high chemoselectivity, convenient synthetic accessibility, and stability of triazole-containing products, enabling the successful implementation of rapid activity screening directly after parallel microplate-scale synthesis without isolation and purification.

Our group reported the rapid discovery of bioactive compounds by employing the miniaturized parallel CuAAC click reaction followed by direct biological screening (**Figure 9**).[52] Specifically, six terminal alkyne fragments were constructed to fully exploit the chemical space within the binding sites. Furthermore, various aryl- and alkyl-substituted azide derivatives were designed to satisfy the needs of structural diversity. The RT inhibition screening of 156-member DAPY derivatives library in 96-well plates identified 22 hits from the compound library, with inhibition rates higher than those of the control drugs at a fixed concentration. Further cell-based biological

evaluation of the 22 hits led to the discovery of **13** as an interesting lead compound with potent anti-HIV activities.

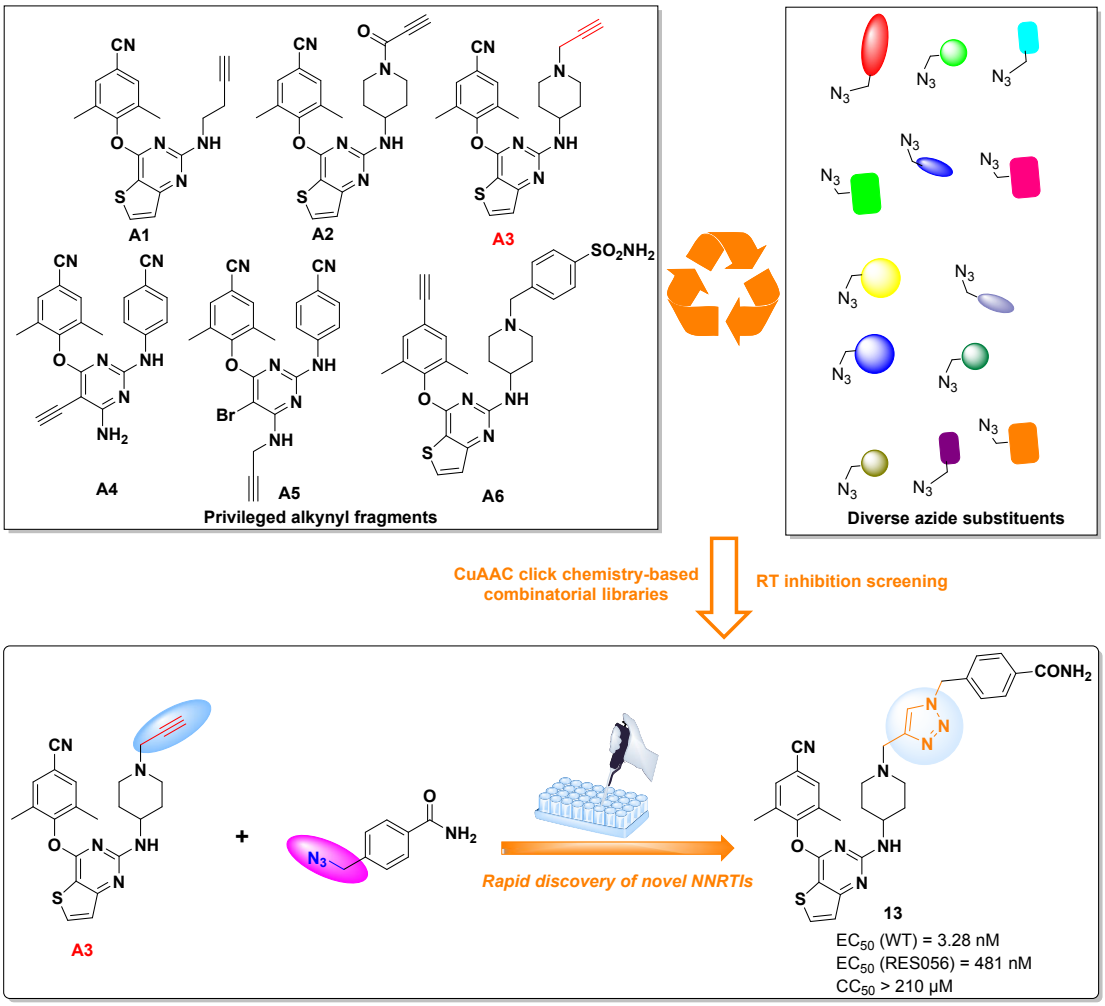


**Figure 9.** The rapid discovery of novel NNRTIs from CuAAC click chemistry-based combinatorial libraries.

## 3. STRUCTURAL OPTIMIZATION OF NNRTI LEADS

To identify promising candidates with both prominent antiviral activities and drug-like properties, classic medicinal chemistry strategies combined with structural biology have been applied in the lead optimization of diverse NNRTIs during recent decades.[53-56]

### 3.1. Scaffold Hopping

The main goal of scaffold hopping is to identify novel compounds containing topologically different backbones by replacing the pivotal structure of existing active compounds by considering key ligand–protein interactions.[57-60]

As the first class of NNRTIs discovered to act on HIV-1 RT specifically, iterative SAR research involving the 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) family has developed promising derivatives **14** (MKC-442) and **15** (TNK-651) that share the uracil scaffold containing an isopropyl group. Recognizing the importance of the bulky isopropyl group in providing sufficient steric hindrance to regulate the conformation of the *ortho*-benzyl moiety to form an optimal π–π interaction with Y181, the replacement of the isopropyl group with various halogens of large atomic radii led to the discovery of iodine-bearing uracil analog **16** with potent activities in RT inhibition and antiviral assays.[61] Encouraged by the robust potencies of these previously discovered compounds, the central uracil scaffold of TNK-651 was converted to a pyridinone ring, and methyl-substituted cyclohexane was introduced to promote the π–σ interaction with the conserved residue W229 rather than the readily mutated residue Y181, which eventually gave rise to compound **17** with an improved resistance profile (**Figure 10**). Then, the **17**-*cis* and **17**-*trans* diastereomers were prepared separately by the Mitsunobu stereoselective reaction to investigate the effect of the 2′-methyl configuration of cyclohexyl on the RT inhibitory potency. The biological results indicated that the activity of **17**-*trans* was approximately 400-fold greater than that of **17**-*cis*.[62]

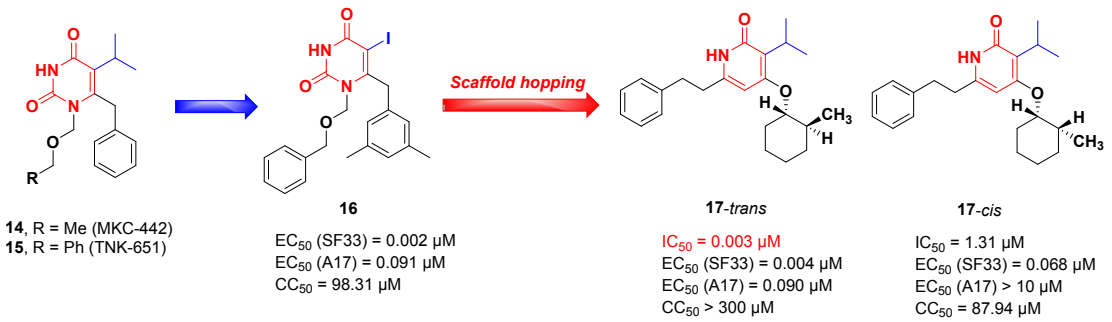


**Figure 10.** Optimization of HEPT-type NNRTIs by employing a scaffold hopping strategy.

A scaffold hopping strategy was also used to optimize the DAPY family to search for novel chemotypes that are structurally different from ETV, which resulted in identification of thiophene[3,2-*d*]pyrimidine derivatives in the hope that the sulfur atom with an electronic nature similar to that of the bromine atom could form strong electrostatic interactions with the surrounding residues (**Figure 11**). Peripheral substituent decoration culminated in the discovery of compound **18**, which contained a benzylsulfamide group with better activity and lower cytotoxicity than ETV, although it was somewhat weaker against RES056.[63] Following the structure-based design, the left cyano group of **18** was changed to a cyanovinyl group to target highly conserved residues in the hydrophobic channel, resulting in compound **19** with overall enhanced potency compared to that of **18**, especially against RES056.[64] Nevertheless, both **18** and **19** (Sol. < 1 μg/mL) were extremely difficult to dissolve owing to the introduction of aromatic rings. The cocrystal structures of **18** (**Figure 12A**) and **19** (**Figure 12B**) with RT illustrated that the conformational flexibility of molecules, multiple hydrophobic contacts, and the main chain hydrogen-bonding network explain their highly effective activities. Afterward, the thiophene ring was replaced with various aliphatic rings, resulting in the exceptionally potent dihydrofuro[3,4-*d*]pyrimidine derivative **20**, which

showed improved solubility.[65] Additionally, **20** showed favorable safety and PK profiles with moderate bioavailability and a long half-life (**Table 2**). Recently, a series of trisubstituted pyrimidine derivatives were discovered by opening the fused ring of lead compound **18**.[66] Among them, the most promising compound **21**, bearing a pyridyl-substituted pyrimidine scaffold, exhibited improved resistance profiles and reduced cytotoxicity compared to ETV. In addition, **21** showed favorable safety properties and PK profiles with a moderate oral bioavailability of 15.3% *in vivo*. The cocrystal structures of RT/**21** indicated the critical role of the hydrogen bond network mediated by water molecules around NNIBP in binding and resisting residue mutations (**Figure 12C**).

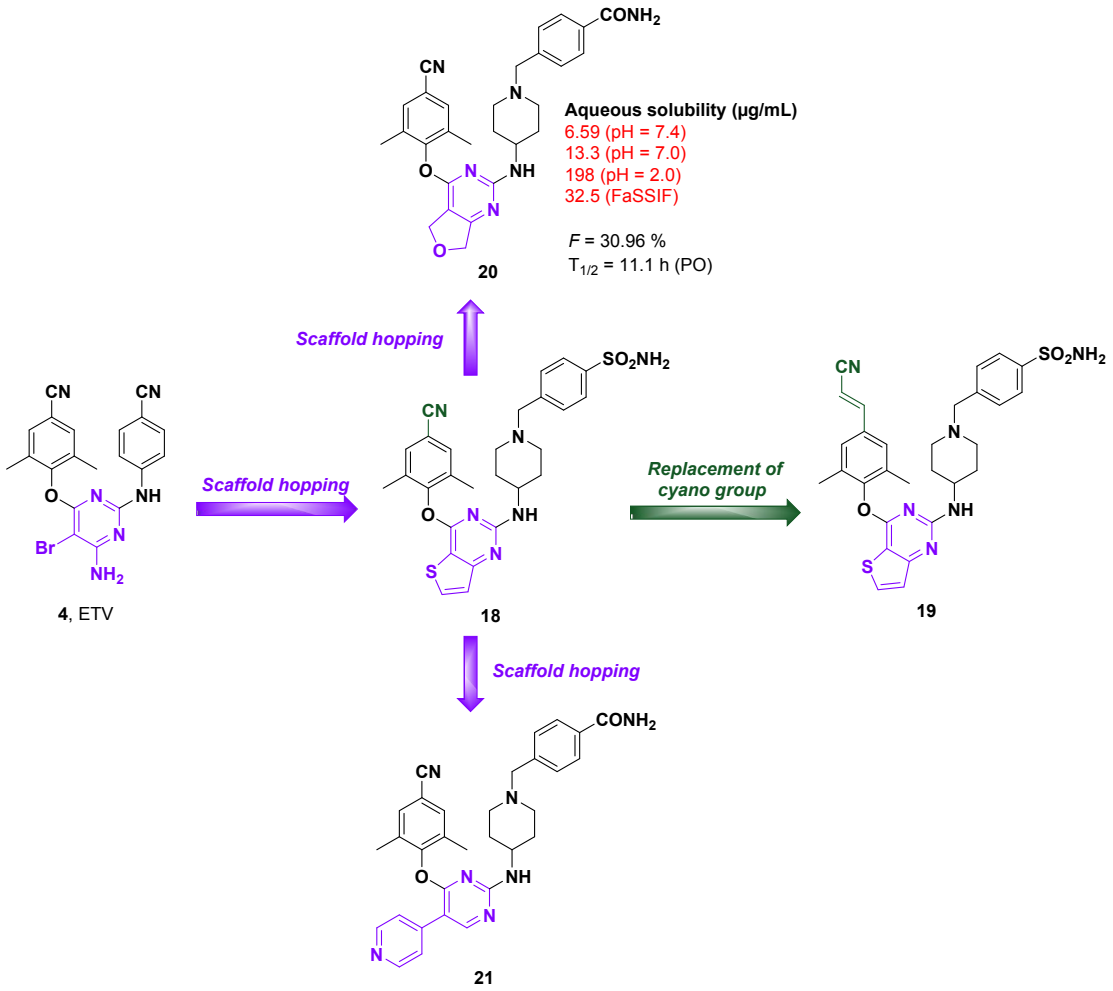

**Figure 11.** Development of DAPY-based NNRTIs *via* a scaffold hopping strategy in our group.

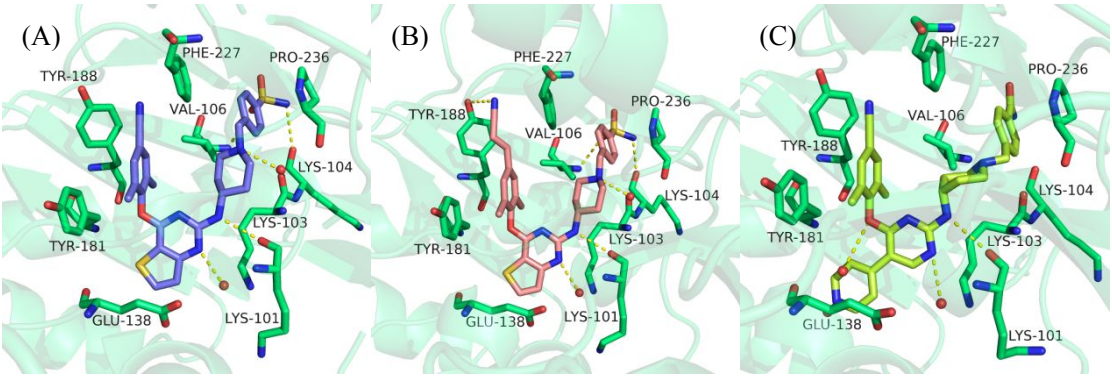


**Figure 12.** (A) Cocrystal structures of compound **18** (blue) and WT RT (PDB code: 6C0J); (B) Cocrystal structures of compound **19** (pink) and WT RT (PDB code: 6C0N); (C) Cocrystal structures of compound **21** (yellow) and WT RT (PDB code: 7KWU). Hydrogen bonds are shown as yellow dotted lines.

**Table 2.** Antiviral Activity and Cytotoxicity of Compounds **18−21**.

| Compd | $EC_{50}$ (nM)[a] | | | | | | | | $CC_{50}$ (μM)[b] |
|---|---|---|---|---|---|---|---|---|---|
| | WT | L100I | K103N | Y181C | Y188L | E138K | F227L/V106A | K103N/Y181C | |
| **18** | 1.4±0.4 | 3.4±0.6 | 2.9 | 3.2±0.4 | 3.0±0.1 | 2.9 | 4.2±1.2 | 30.6±12 | >227 |
| **19** | 1.22±0.26 | 1.34±0.50 | 0.96±0.07 | 5.00±0.11 | 5.45±0.20 | 4.74±0.16 | 2.70±1.74 | 5.50±0.81 | 2.30±0.47 |
| **20** | 1.6±0.4 | 2.4±0.2 | 0.9±0.1 | 4.4±1.1 | 8.4±3.6 | 7.0±2.5 | 19.0±2.2 | 41.5±9.9 | >250 |
| **21** | 3.75±0.40 | 4.26±0.62 | 3.79±0.42 | 6.79±1.49 | 6.79±2.82 | 10.9±5.63 | 10.4±5.30 | 24.4±3.06 | 36.0±4.85 |
| **EFV**[63] | 5.03±2.12 | 115±8.28 | 80.1±2.68 | 8.20±1.03 | 514±51.5 | 5.19±0.63 | 348±58.2 | 308±164 | >0.00633 |
| **ETV**[63] | 4.07±0.15 | 5.38±2.06 | 2.35±0.67 | 15.7±2.12 | 20.5±2.92 | 14.4±2.27 | 29.4±7.79 | 17.0±1.78 | >0.00459 |

[a]$EC_{50}$: The $EC_{50}$ value is defined as the dose required to achieve 50% protection of the infected MT-4 cells by the MTT colorimetric method; antiviral curves were established from triplicate samples at each concentration.

[b]$CC_{50}$: The $CC_{50}$ value is defined as the dose required to achieve 50% inhibition of MT-4 cell growth by the MTT colorimetric method; the toxicity curves were established from triplicate samples.

## 3.2. Molecular Hybridization

Molecular hybridization has been widely applied to drug development processes and represents a promising approach to improve binding affinity and overcome cross-resistance.[67-69] However, it is essential to note that hybrids generally have a larger volume of molecules and pose challenges to the prediction of physicochemical properties. Therefore, it is preferred to select the parent molecules with a high degree of overlap and minimize the size of the hybrids as much as possible so that the final combined ligands will not be too large to produce drug-like liabilities.[70]

To remedy the drawbacks of the decreased susceptibility of EFV to the K103N variant and the poor PK profiles of ETV, the hybridization of the chlorophenyl fragment of EFV and the introduction of the cyanovinyl group of RPV led to the discovery of compound **22** by employing the substituent decorating approach on the left-wing (**Figure 13**). Subsequent removal of the chlorine atom from **22** afforded the quinazoline derivative **23** with satisfactory liver microsome stability and PK properties, the potency of which was slightly greater than or comparable to that of ETV and EFV.[71] Alternatively, the superposition of the binding orientations of EFV and ETV motivated the grafting of the 4-cyanoaniline moiety to the carbonyl group of EFV while retaining other essential pharmacophores, leading to the discovery of a series of dihydroquinazolin-2-amine derivatives, as exemplified by compound **24**.[72]

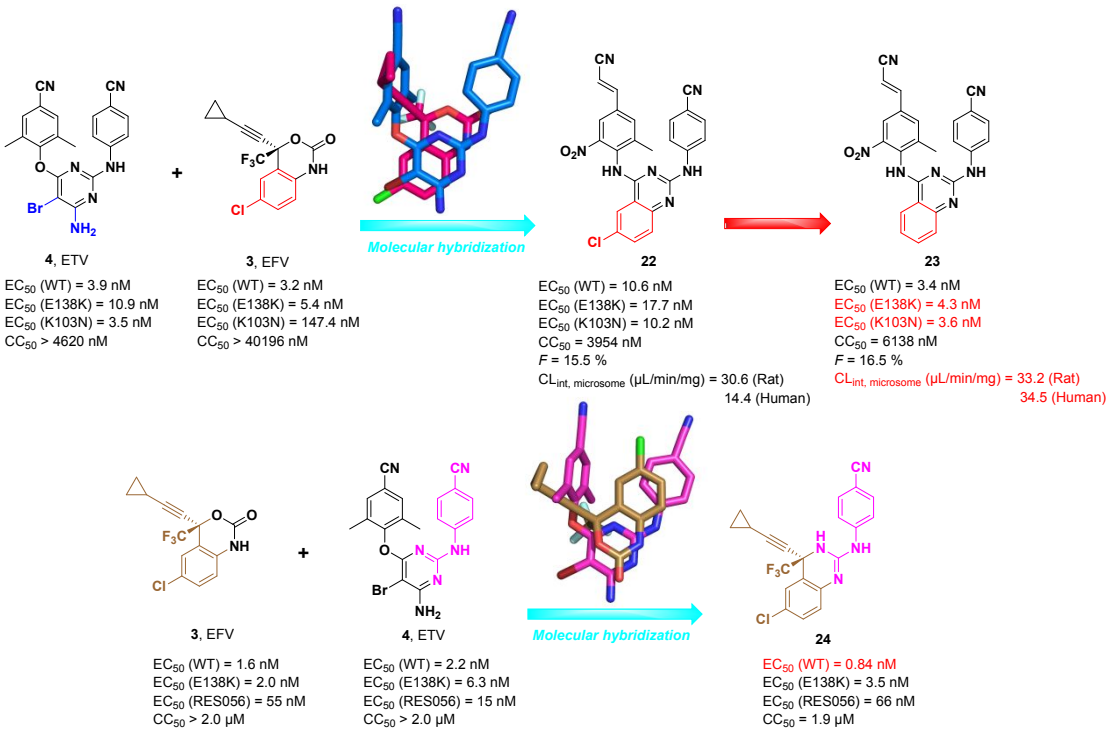


**Figure 13.** Molecular hybridization of the approved NNRTIs ETV and EFV. Overlay of cocrystal structures of ETV (**4**, blue) and EFV (**3**, red) with WT RT. Overlay of cocrystal structures of EFV (**3**, brown) and ETV (**4**, magenta) with WT RT (PDB codes: 1FK9 and 3MEC).

As shown in **Figure 14**, the discovery of doravirine began with the hit compound tetrazole thioacetanilide derivative **25**, which was identified *via* a high-throughput screening. However, **25** proved to be incapable of being orally bioavailable and was rapidly metabolized to the carboxylic acid form in rat plasma. Subsequent extensive SAR explorations were conducted by the combination of more stable substituents both on the anilide and the benzene linked to the tetrazole core, which resulted in compound **26**, with improved oral bioavailability and decreased plasma clearance.[73] Nonetheless, suboptimal PK results of the tetrazole thioacetanilide analogs prompted the evolution of the new scaffold.[74, 75] Inspired by the overlap of the arylacetamide structures of compound **26** and the known NNRTI candidate **27** (GW678248),[76-79] a novel class of

diaryl ethers was obtained *via* the molecular hybridization of **26** and **27** to optimize interactions with the binding site. These diaryl ether phenoxyacetanilide derivatives (exemplified by compound **28**) were rapidly eliminated *in vivo* because of the occurrence of metabolic hydrolysis of the aniline moiety. Hence, a variety of isosteric heterocycles were introduced to replace the metabolizable aniline group, yielding the indazol-containing compound **29**.[80] However, the poor solubility and low bioavailability ($F$ = 3.3%) of **29** hindered its further development. Since the crystallographic studies indicated that the lower edge of the indazole ring was located at the protein−solvent interface, subsequent efforts have focused on replacing the phenyl moiety of the indazolyl group with a solubilizing pyridyl moiety to improve the physicochemical properties. Further refinement resulted in the identification of **30**, which was labeled MK-4965, as a drug candidate, exhibiting excellent activity against various HIV-1 strains and improved bioavailability ($F$ = 52%).[81, 82]

The follow-up optimizations adopt the methylene linker of another compound **31**,[83-85] in which the side chain was truncated and a pyridone platform was introduced instead of the *meta*-resorcinol moiety, leading to 4-chloro pyridone compound **32** with superior anti-resistance profiles.[86] To improve the exposure and plasma half-life after oral administration of **32** ($T_{1/2}$ = 2.2 h), the tolerant exploration of various small lipophilic substituents (such as alkyl, halogen, and fluorinated alkyl) at the 4-position of pyridone led to the discovery of 4-trifluoromethyl analog **33** with improved plasma stability and a prolonged half-life ($T_{1/2}$ = 7.0 h). Owing to the strong intermolecular hydrogen bonds of the pyrazolopyridyl moiety, compound **33** suffers from poor

solubility (Sol. = 1.1 μM) and oral bioavailability ($F$ = 15%).[87] In parallel with this effort, the bioisosteric replacement of pyrazolopyridine was also conducted to disrupt intermolecular interactions, resulting in the discovery of methyltriazolinone compound **6** (MK-1439, doravirine) with improved solubility (Sol. = 45 μM) and a favorable pharmacokinetic profile ($F$ = 57%).[87]

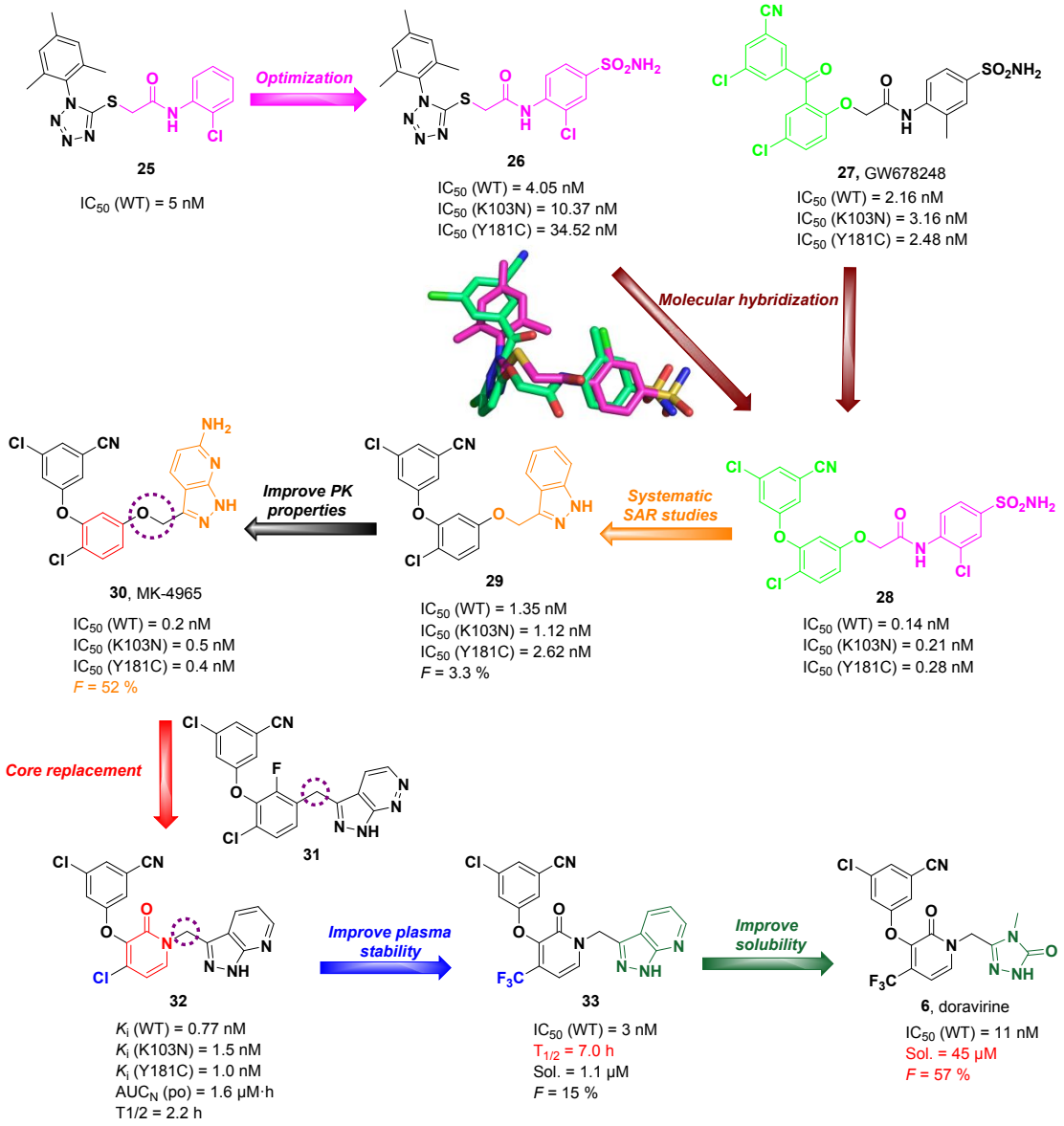


**Figure 14.** Illustration of the development process of doravirine. Overlay of the cocrystal structures of compound **26** (magenta) and GW678248 (**27**, green) with K103N RT (PDB code: 3DOK).

Given the current landscape of available antiretroviral agents for HIV-1 infections,

the development of novel and highly effective NNRTIs is an arduous campaign. Many positive attributes of the NNRTI class, such as high potency and long plasma half-life, make them attractive targets for drug discovery. However, more than two decades of optimization attempts on NNRTIs have failed to provide ideal clinical efficacy compared with existing treatment options. DOR has become the only NNRTI that has successfully obtained U.S. FDA approval in recent years.[88-90]

The emergence of DOR alleviates the known deficiencies related to other drugs, including the reduced susceptibility to the mutant strains K103N, Y181C, and G190A that are associated with more than 90% of NNRTI resistance; the neuropsychiatric side effects of EFV; and the cardiotoxicity, food restriction, and high viral load limitation of RPV. In addition, DOR exhibits a more favorable drug−drug interaction profile than earlier NNRTIs, as it does not affect the expression of CYP or other major drug-metabolizing enzymes or drug transporters. The long half-life of DOR allows for its once-daily administration as a single tablet or the fixed-dose combination.[91-93]

Overall, the complementary advantages of structural biology, *in vitro* resistance characterization, and multiple medicinal chemistry strategies facilitated the discovery of DOR with distinct anti-resistance profiles and good safety properties.[94, 95] Currently, the most recent DHHS guidelines recommend the use of DOR-containing therapy under specific treatment conditions due to the lack of sufficient clinical experience. DOR is expected to be reintroduced as a first-line treatment option on behalf of the NNRTI class as a result of the accumulation of clinical experience and positive patient outcomes.

### 3.3. Bioisosteric Replacement

The bioisosteric replacement strategy allows the adjustment of the physicochemical properties while retaining their efficient target engagement. Classic bioisosteres with the same atomic number are required to be isoelectronic; otherwise, nonclassical bioisosteres are likely to differ significantly in the number of atoms and valence electrons but induce similar biological activities.[96-98]

The replacement of the pyrimidine moiety of ETV with an aminobenzene moiety to compensate for the key hydrogen-bonding interactions between pyrimidine and K101 led to the identification of the potent diarylaniline derivative **34**.[99] Then introduction of the cyanovinyl group at the cyano position of **34** for insertion into the hydrophobic region resulted in a more active compound **35** with subnanomolar $EC_{50}$ values.[100] Thereafter, to overcome the poor solubility and low permeability of **35**, compound **36** was discovered by allowing substituent modifications at the cyanovinyl and nitro positions of **35**. As expected, the incorporation of the hydroxymethyl group could promote the dissolution of **36**, which was far more soluble than **35** and equivalent to RPV.[101] However, due to the relatively low metabolic stability and high clearance rate of **36**, subsequent optimization was carried out to maintain a balance between anti-HIV activity and multiple physicochemical properties; this effort culminated in compound **37**, which showed significantly improved solubility and metabolic stability compared to RPV while maintaining excellent antiviral activity (**Figure 15**).[102]

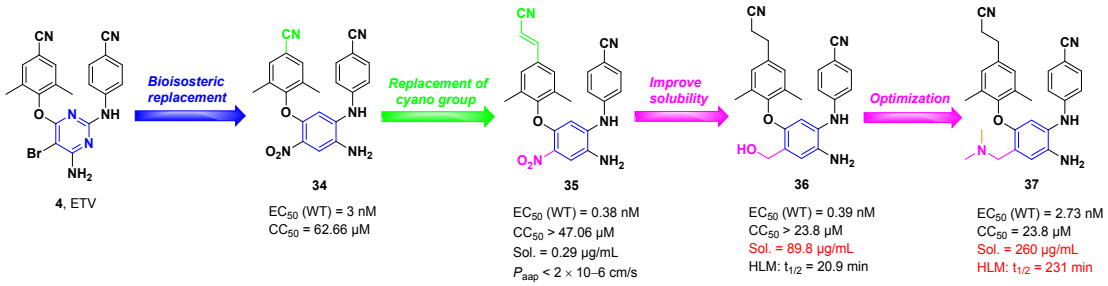


**Figure 15.** Optimization of novel diarylaniline derivatives.

With **19** as the lead compound, novel thiophene[2,3-*d*]pyrimidine derivatives were identified *via* the structure-based bioisosterism strategy to reduce the toxicity and improve the druggability profile (**Figure 16**).[103] Compared to **19**, the most potent compound **38** showed comparable activities and much better drug-like properties (**Table 3**). In addition, it was obvious that the RT inhibitory activities ($IC_{50}$) of **19** and **38** differ significantly from their antiviral activities ($EC_{50}$), possibly due to the distinct testing methods of the two biological evaluation screening systems (**Table 4**). In the enzyme inhibition assay, the RT inhibitory activities are related to the amount of biotin deoxyuridine triphosphate and RT. In the anti-HIV-1 assay, the antiviral activities are related to the amount of MT-4 cells and HIV-1, the replication round, and the serum protein used to indicate the protein binding of test compounds. Notably, the HIV-1 WT strain was passaged in MT-4 cell culture with increasing concentrations of compound **38** to select for HIV-1 mutant strains. Furthermore, no phenotypic cross-resistance toward NRTIs was observed in the selected mutations that were resistant to **38**.

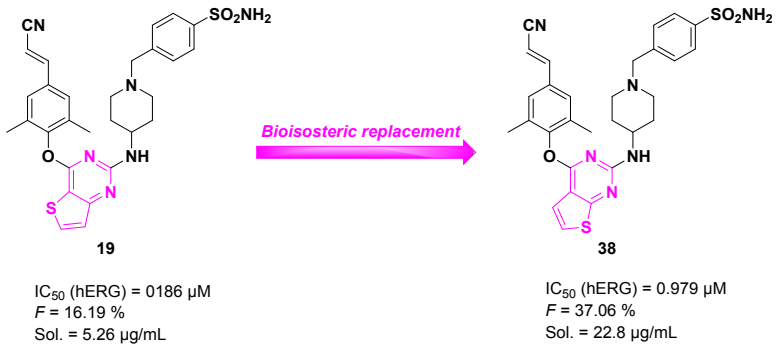


**Figure 16.** Bioisosteric replacement of the thiophene[3,2-*d*]pyrimidine compound **19**.

**Table 3.** Antiviral Activity and Cytotoxicity of Compound **38**.

| Compd | $EC_{50}$ (nM)[a] | | | | | | | | $CC_{50}$ (μM)[b] |
|---|---|---|---|---|---|---|---|---|---|
| | WT | L100I | K103N | Y181C | Y188L | E138K | F227L/V106A | K103N/Y181C | |
| **38** | 3.24±2.15 | 2.05±0.58 | 2.34±0.82 | 6.57±0.33 | 7.59±0.46 | 6.70±0.49 | 4.81±0.84 | 6.45±0.49 | 10.1±3.28 |
| **19** | 1.22±0.26 | 1.34±0.50 | 0.96±0.07 | 5.00±0.11 | 5.45±0.20 | 4.74±0.16 | 2.70±1.74 | 5.50±0.81 | 2.30±0.47 |
| **RPV**[103] | 1.00±0.27 | 1.54±0.00 | 1.31±0.36 | 4.73±0.48 | 79.4±0.77 | 5.75±0.11 | 81.6±21.2 | 10.7±7.96 | 3.98 |
| **ETV**[103] | 4.07±0.15 | 5.38±2.06 | 2.35±0.67 | 15.7±2.12 | 20.5±2.92 | 14.4±2.27 | 29.4±7.79 | 17.0±1.78 | >4.59 |

[a]$EC_{50}$: The $EC_{50}$ value is defined as the dose required to achieve 50% protection of the infected MT-4 cells by the MTT colorimetric method; antiviral curves were established from triplicate samples at each concentration.

[b]$CC_{50}$: The $CC_{50}$ value is defined as the dose required to achieve 50% inhibition of MT-4 cell growth by the MTT colorimetric method; the toxicity curves were established from triplicate samples.

**Table 4.** Inhibitory Activity against WT and Mutant RT of Compound **38**.

| Compd | $IC_{50}$ (μM)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | WT | L100I | K103N | Y181C | Y188L | E138K | F227L/V106A | K103N/Y181C |
| **38** | 0.114±0.029 | 0.228±0.090 | 0.222±0.010 | 0.141±0.003 | 0.452±0.083 | 0.374±0.203 | 0.130±0.013 | 0.120±0.057 |
| **19** | 0.101±0.015 | 0.099±0.040 | 0.191±0.022 | 0.149±0.005 | 0.301±0.071 | 0.350±0.120 | 0.106±0.040 | 0.107±0.052 |
| **RPV**[103] | 0.015±0.001 | 0.024±0.012 | 0.027±0.000 | 0.021±0.002 | 0.084±0.038 | 0.041±0.021 | 0.015±0.004 | 0.023±0.014 |
| **ETV**[103] | 0.012±0.002 | 0.013±0.004 | 0.025±0.002 | 0.017±0.002 | 0.046±0.009 | 0.032±0.011 | 0.008±0.002 | 0.019±0.008 |

[a]$IC_{50}$: The $IC_{50}$ value is defined as the concentration required to achieve 50% inhibition of the incorporation of biotin deoxyuridine triphosphate (biotin-dUTP) into HIV-1 RT.

### 3.4. Conformational Restriction

Conformational restriction of ligand flexibility has attracted considerable attention in novel drug discovery, as it can enhance activity by reducing entropic loss during ligand–receptor binding and by stabilizing the preferential conformation, as well as improve metabolic stability by introducing specific structural constraints to block metabolically sensitive sites.[104, 105]

The distinct conformation of the oxymethylene linker presented in the cocrystal structures of **30** (MK-4965) prompted exploration of the functionality of conformational restriction (**Figure 17**). Therefore, cyclization of the central benzene ring with five-membered aromatic heterocycles was performed to improve the metabolic stability by weakening the oxidative dealkylation of the pyrazolopyridyl group; this campaign resulted in the confirmation of indazolyl as the best cyclic substituent and generated MK-6186 (**39**) and MK-7445 (**40**) with a good combination of antiviral activity and PK profile.[106]

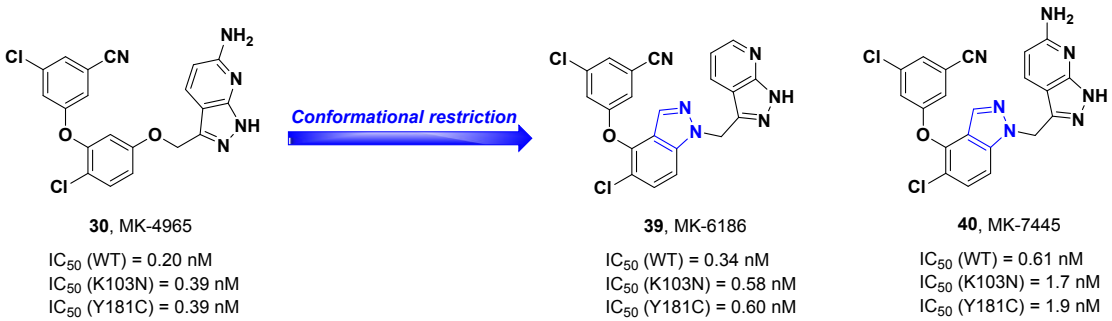


**Figure 17.** Optimization of MK-4965 *via* a conformational restriction approach.

The application of conformational restriction strategies was also observed in the research process of DAPY derivatives (**Figure 18**). DAPYs are characterized by torsional flexibility ("wiggling") and repositioning ("jiggling") due to the rotatable *O*

or *NH* linker on both sides of the central core. The cyclization of the pyrimidine ring with the left or right NH group of RPV by introducing the fused ring structure afforded the potent 2,9-purine analogs **41** and **42**.[107, 108]

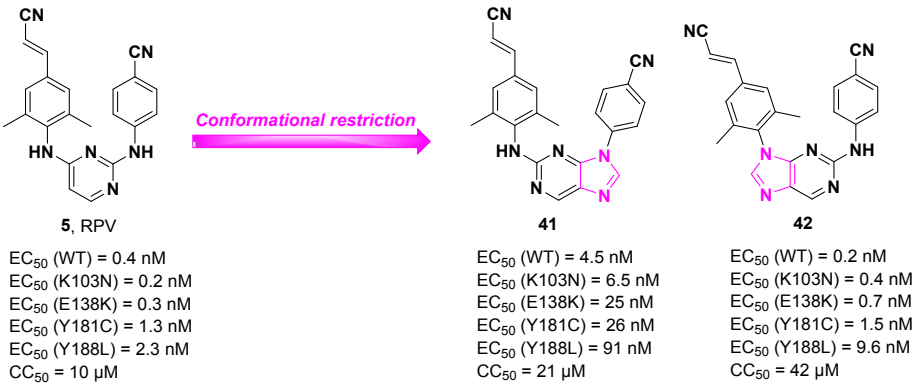


**Figure 18.** Discovery of novel conformationally restricted DAPY derivatives.

Conformational restriction can often lead to improved potency and decreased drug metabolism, but inherent conformational flexibility is critical to the favorable anti-resistance profile of NNRTIs.[109-111] Notably, the inherent flexibility of NNRTIs enables them to continually adjust their conformation in the NNIBP, contributing to maintaining binding affinity for various resistant RT mutations.[112-114] Therefore, the trade-off between conformational flexibility and restriction appears to be particularly important in the application of the conformational restriction strategy for the optimization of NNRTIs. After all, only a suitable conformation that matches well with the binding pocket can produce the optimal potency.

## 4. MEDICINAL CHEMISTRY STRATEGIES TO OVERCOME DRUG RESISTANCE

Drug resistance causes major challenges for drug design against mutated proteins

and impedes the performance of existing therapies in clinical practice. Due to the lack of mechanisms for detecting and repairing potential base mismatches, the frequent emergence of HIV-1 resistant mutations against approved antiretroviral drugs necessitates the continued search for novel NNRTIs by utilizing alternative drug design strategies.[115-117] Although resistance may not be avoided entirely, designing new inhibitors based on the medicinal chemistry strategies discussed here could alleviate the prevalence of drug resistance.[118]

**4.1. Covalent Binding Strategy**

Covalent inhibitors exert their biological functions by reacting irreversibly or reversibly with nucleophilic groups of proteins.[119-121] The covalent binding strategy enables the discovery of highly efficient, selective, and long-acting chemical molecules.

Y181C substitution is the most prevalent mutation after treatment with NNRTIs, especially for NVP and EFV. To address this problem, novel NNRTIs targeting the Y181C mutation were discovered *via* the covalent binding design strategy (**Figure 19**). By analyzing the cocrystal structures of **11** and WT RT (**Figure 7A**), the chlorine atom on the naphthalene ring of **11** points to the amino acid at position 181.[122] Using **11** as a lead compound, two types of relatively less reactive electrophilic substituents were chosen to replace the chlorine atom, including haloamides and acrylamides. The inhibitory ability of chloromethylamide **43** and the acrylamide **44** on variants containing Cys181 presented a time-dependent characteristic; that is, the potency was enhanced with increasing incubation time. Notably, after 48 h of incubation, the $IC_{50}$ values of compounds **43** and **44** against the variants were in the range of 0.14 to 0.19

μM, approximately 10 times lower than that against the WT strain. Time-dependent inhibition kinetic analyses confirmed that compound **44** completely suppressed the catalytic activity of Y181C RT after 72 h due to irreversible covalent binding. In addition, mass spectrometry experiments revealed a single shifted peak on the p66 subunit of Y181C RT, and the added value was fully consistent with the molecular weight of the corresponding inhibitors. Most importantly, the covalent bond between the electrophilic warhead and Cys181 was visible in the cocrystal structures of **43** or **44** and Y181C RT (**Figure 20**).

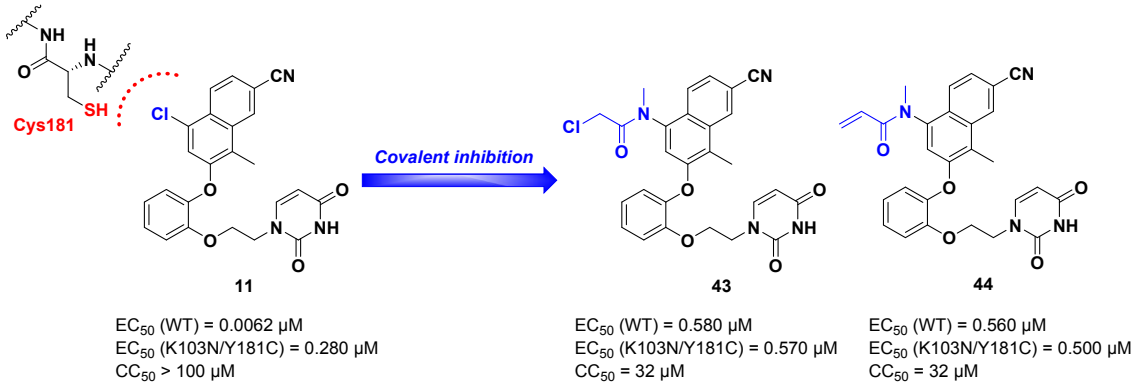


**Figure 19.** Identification of novel 2-naphthyl catechol diether derivatives as covalent NNRTIs by targeting Cys181.

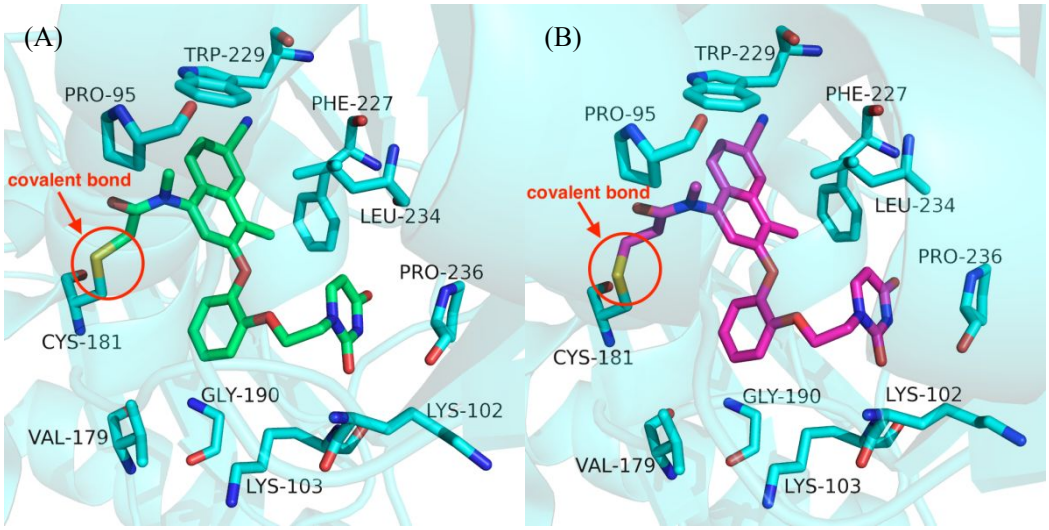


**Figure 20.** (A) Cocrystal structures of compound **43** (green) and Y181C RT (PDB code: 5VQX).

(B) Cocrystal structures of compound **44** (magenta) and Y181C RT (PDB code: 5VQV). The covalent bond is shown as a yellow line.

Distinct from the covalent modification of the Y181C mutant protein, the design of covalent inhibitors targeting WT RT seems more complicated due to the lack of cysteine. Although NNIBP contains numerous lysine residues, they are located in the solvent-exposed region and do not have sufficient nucleophilicity. In contrast, tyrosine residues become preferred through potential covalent binding of the side chain phenolic hydroxyl. Therefore, based on the cocrystal structures of **8** and WT RT (**Figure 4**), a series of fluorosulfate-bearing derivatives of compound **8** were designed in light of the stability of fluorosulfate and its relatively modest electrophilicity (**Figure 21**). As a result, compounds **45−47** were identified as covalent inhibitors by the crystallographic studies.[123] As shown in **Figure 22**, these compounds covalently modified Tyr181 to form biaryl sulfate between tyrosine and the fluorosulfate group. Generally, the inhibitory potency of covalent inhibitors improves with the extension of the incubation time, and non-covalent inhibitors are not affected. However, the known covalent inhibitors **45−47** did not show a typical time-dependent effect, while the potency of other non-covalent inhibitors decreased significantly over time. The tested inhibitor may have been partially degraded during the assay, as indicated by the decreasing potency of the non-covalent inhibitor, but this effect was counteracted by the irreversible binding of the covalent inhibitor to the enzyme. Moreover, the activity of all of these compounds was substantially inferior to that of compound **8**, presumably because the fluorosulfate group with increased bulk and polarity caused steric conflict

with Lys101 and weakened the binding affinity. Notably, mass spectrometry was used to confirm the covalent modifications, and an accurate mass increase was observed only for the p66 catalytic subunit.

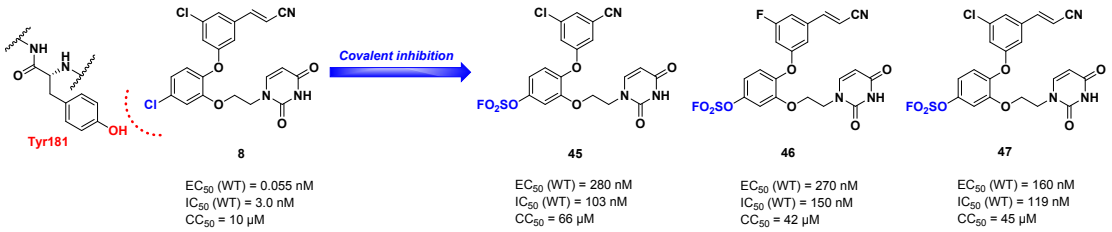


**Figure 21.** Identification of novel fluorosulfate-bearing derivatives as covalent NNRTIs by targeting Tyr181.

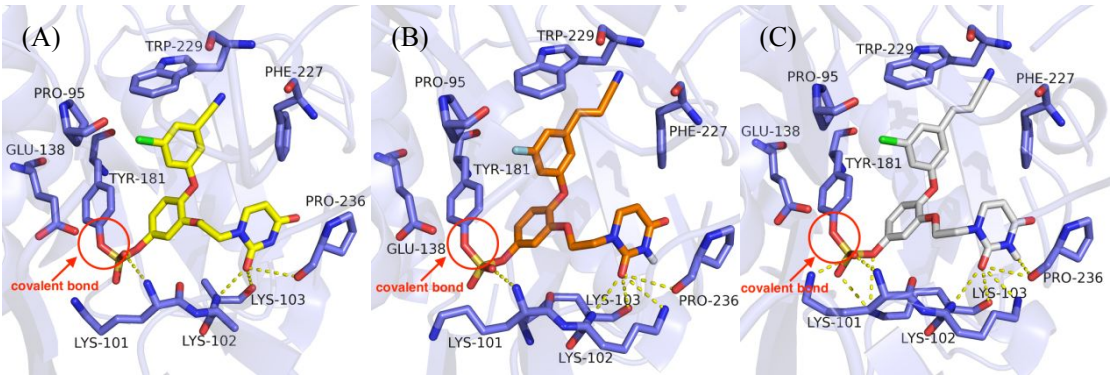


**Figure 22.** (A) Cocrystal structures of compound **45** (yellow) and WT RT (PDB code: 7KRD). (B) Cocrystal structures of compound **46** (orange) and WT RT (PDB code: 7KRF). (C) Cocrystal structures of compound **47** (white) and WT RT (PDB code: 7KRC). The covalent bond is shown as a red line.

## 4.2. Targeting Highly Conserved Regions

Analysis of the nature of the amino acids in the binding site revealed that mutations occurred less frequently on highly conserved residues. Therefore, designing inhibitors that specifically interact with highly conserved residues while reducing the dependence on potentially variable residues holds promise as an alternative strategy for the treatment of constantly emerging mutants.[124, 125]

The cyanovinyl moiety of RPV extends into the hydrophobic channel formed by residues Tyr181, Tyr188, Phe227, and Trp229, contributing to an improvement in drug resistance profiles. However, the electrophilic cyanovinyl group may lead to potential covalent modification and high cytotoxicity as a Michael acceptor.[126] Therefore, to extend the left-wing conjugation system, diverse biphenyl moieties were introduced to increase the degree of aromaticity and stretch into the hydrophobic channel to strengthen interactions with the highly conserved region (**Figure 23**); this work resulted in the discovery of compound **48** bearing a 3,5-dimethyl-[1,1'-biphenyl]-4'-carbonitrile substituent, which could strongly suppress the whole panel of viral strains.[127] Afterward, a series of nondimethylphenyl DAPYs were identified to overcome the high cytotoxicity of **48**, and the most active 3,5-difluoro biphenyl derivative **49** showed prominent inhibitory activity and no apparent cytotoxicity.[128] Further addition of a methyl group to the central scaffold yielded compound **50** with better *in vitro* metabolic stability.[129] In addition, a variety of aromatic heterocycles were introduced on the terminal benzonitrile of **49** by using the fragment-based replacement strategy, leading to the identification of compound **51** bearing a pyridinyl group with excellent druggability (**Table 5**).[130]

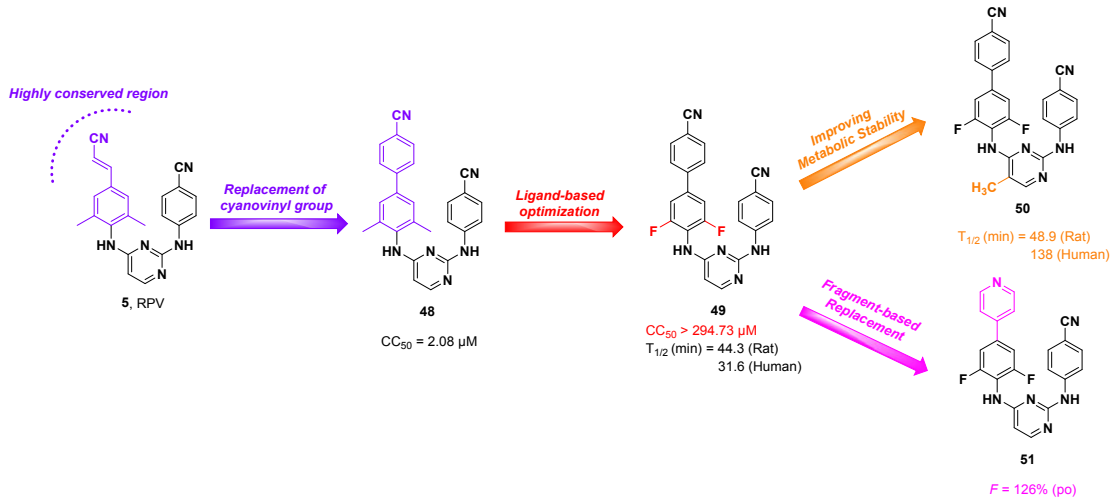


**Figure 23.** Development of DAPY-type NNRTIs bearing a biphenyl moiety.

**Table 5.** Antiviral Activity and Cytotoxicity of Compounds **48**−**51**.

| Compd | $EC_{50}$ (nM)[a] | | | | | | $CC_{50}$ (μM)[b] |
|---|---|---|---|---|---|---|---|
| | WT | L100I | K103N | Y181C | Y188L | E138K | |
| **48** | 1±0.6 | 1.3±0.1 | 0.84±0.66 | 1.5±0.0 | 11±1 | 2±0.6 | 2.08±0.72 |
| **49** | 1.3±0 | 10.8±5 | 2.6±0 | 6.1±1 | 130±12 | 1.9±1 | >294.73 |
| **50** | 1±0.32 | 16.3±5.3 | 5.4±1.8 | 11.2±1.9 | - | 8.3±1.5 | >285 |
| **51** | 1.0±0 | 20.4±3.7 | 8±1.1 | 15±0.9 | 417±39 | 4±1.8 | >313 |
| **EFV**[130] | 4±1.5 | 44.7±12.6 | 91.5±23.6 | 5.4±1.2 | 269±31.2 | 7.5±0.54 | >6.33 |
| **ETV**[130] | 4±0.68 | 10.7±4.6 | 3.4±0.34 | 18.9±5.9 | 29.3±9.3 | 17.5±7.7 | >4.60 |

[a]$EC_{50}$: The $EC_{50}$ value is defined as the dose required to achieve 50% protection of the infected MT-4 cells by the MTT colorimetric method; antiviral curves were established from triplicate samples at each concentration.

[b]$CC_{50}$: The $CC_{50}$ value is defined as the dose required to achieve 50% inhibition of MT-4 cell growth by the MTT colorimetric method; the toxicity curves were established from triplicate samples.

## 4.3. Targeting an Additional Binding Site

According to the classic concept of receptor–ligand interactions, an efficient NNRTI should interact with NNIBP and additional binding sites within RT as much as possible. The extraordinary advantage of this strategy lies in the interactions of the

molecule with other sites that can compensate for the decreased affinity caused by specific amino acid mutations at one binding site, ensuring that the inhibitor is less sensitive to variants.[131, 132] Here, we demonstrated the application of this design strategy in the following representative cases.

The NNRTI-adjacent binding site spanning the palm and connection domains was identified as an unexploited new site approximately 14.46 Å from the NNIBP.[133] Based on crystallographic studies, our group initially attempted to design dual-site NNRTIs occupying both the NNIBP and NNRTI-adjacent binding sites (**Figure 24**). With ETV as the lead compound, diverse substituents varying in volume and electronic nature were incorporated into the sulfur atom or sulfone group of the central scaffold through different linkers to fully explore the chemical space of the NNRTI-adjacent binding site (**Figure 25**). Notably, the most potent compound **52** bearing a morpholine ring showed superior activity and decreased cytotoxicity compared with ETV.[134] In the follow-up optimization work, olefinic bonds with steric positioning advantages were creatively introduced to ensure that the terminal moiety accurately stretched to the NNRTI-adjacent binding site, as exemplified by the two most effective compounds **53** and **54** (**Table 6**).[135]

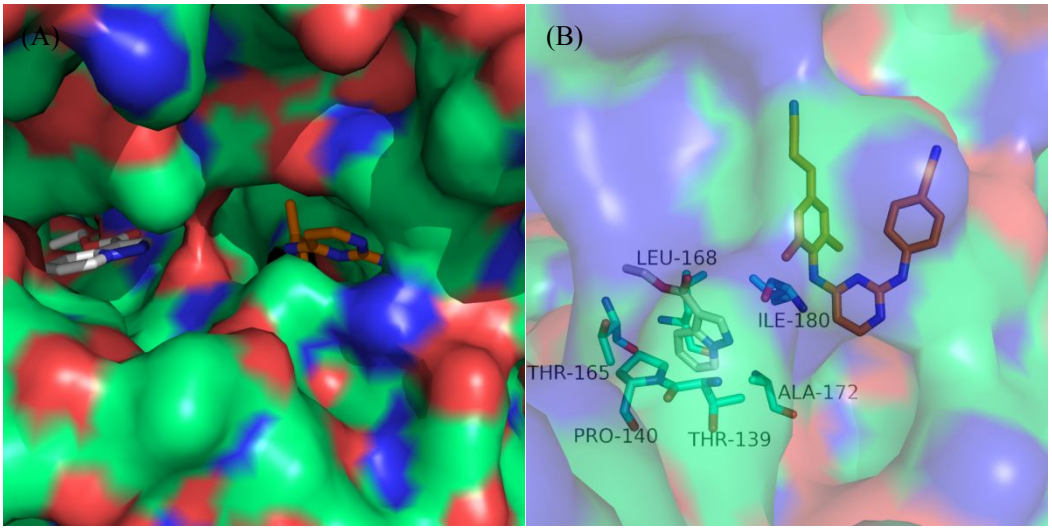


**Figure 24.** (A) Protein/solvent interface of NNIBP and the NNRTI-adjacent binding pocket (PDB code: 4KFB). (B) Cocrystal structures of RPV and the NNRTI-adjacent fragment with WT RT (PDB code: 4KFB).

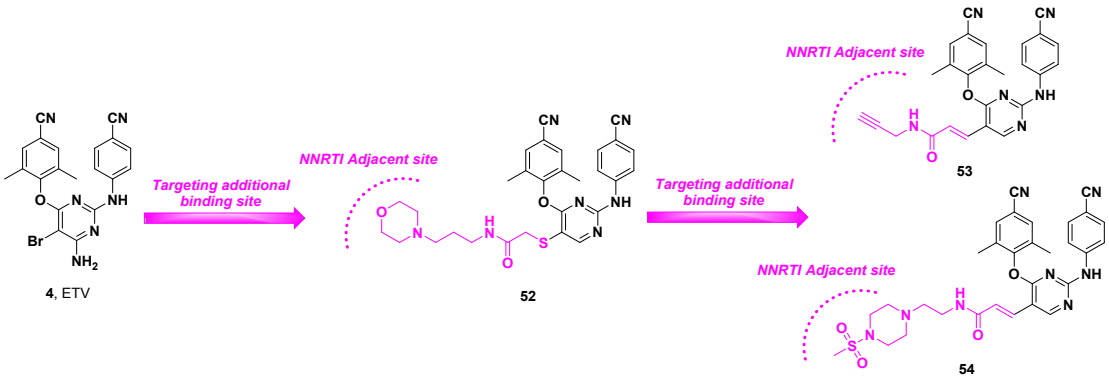


**Figure 25.** Design of novel NNRTIs by targeting the NNIBP and NNRTI-adjacent binding sites.

**Table 6.** Antiviral Activity and Cytotoxicity of Compounds **52−54**.

| Compd | $EC_{50}$ (nM)[a] | | | | | | | | $CC_{50}$ (μM)[b] |
|---|---|---|---|---|---|---|---|---|---|
| | WT | L100I | K103N | Y181C | Y188L | E138K | F227L/V106A | K103N/Y181C | |
| **52** | 2.6±1.1 | 6.5±2.2 | 1.4±0.3 | 11.6±1.0 | 16.2±2.6 | 6.0±0.6 | 105.9±20.9 | 345.2±69.7 | 27.2±4.6 |
| **53** | 5.20±1.50 | 113±33.4 | 10.4±0.60 | 61.8±13.2 | 64.5±15.6 | 10.6±3.00 | 402±313 | 1291±290 | 141±37.7 |
| **54** | 6.10±1.30 | 78.6±31.4 | 8.70±3.10 | 48.6±13.1 | 101±24.3 | 20.9±0.80 | 251±91.7 | 808±180 | 5.90±0.800 |
| **EFV**[134] | 5.2±0.9 | 81.0±16.6 | 98.8±24.4 | 6.7±1.4 | 370.8±43.9 | 6.3±2.4 | 218.5±85.1 | 225.4±113.2 | >6.3 |
| **ETV**[134] | 4.0±0.3 | 20.1±7.29 | 3.3±0.6 | 17.6±4.5 | 34.1±12.2 | 16.9±10.0 | 27.6±3.0 | 35.0±12.9 | 2.2±0.1 |

[a]$EC_{50}$: The $EC_{50}$ value is defined as the dose required to achieve 50% protection of the infected MT-4 cells by the

MTT colorimetric method; antiviral curves were established from triplicate samples at each concentration.

[b]$CC_{50}$: The $CC_{50}$ value is defined as the dose required to achieve 50% inhibition of MT-4 cell growth by the MTT colorimetric method; the toxicity curves were established from triplicate samples.

As an important complement to NNRTIs, NRTIs cause chain termination during DNA strand elongation rather than inhibit polymerase activity. Notably, the proximity of the respective NRTI and NNRTI binding sites enabled the simultaneous occupation of two sites and the rationality of developing novel chimeric inhibitors to achieve the synergistic inhibition of RT. Based on the available structural biology information, chimeric NRTI/NNRTI bifunctional inhibitors connected by a flexible joint were designed, and the positions of the anchor and the optimal length of the connecting linker were determined with the aid of computer modeling (**Figure 26**). Specifically, the 2′,3′-didehydrothymidine (d4T) was selected as an NRTI given its high binding affinity to RT comparable to that of natural analogs. Accordingly, the conformational flexibility of DAPY-type NNRTI (TMC-derivative) with retained basic pharmacophores was utilized to accommodate the addition of the conjugate moiety. On the other hand, a flexible PEG linker was used to connect the two moieties because of its favorable water solubility and significant lipophilicity. Earlier efforts on chimera inhibitors demonstrated that most of them are inactive, probably due to the failure of phosphorylation of the nucleoside portion catalyzed by cellular kinases. Therefore, the metabolically active triphosphate form was prepared directly in this work to bypass the prerequisite pathway for catalysis to the phosphorylation requirement. Incorporation assays indicated that the triphosphate form compound 4TTP-4PEG-TMC (**55**) could

bind to the active site and be incorporated as a substrate in a base-specific manner by HIV-1 RT. In the biological evaluation *via* a steady-state competition assay, **55** displayed the best activity against HIV-1 RT ($IC_{50}$ = 3 nM), which was approximately 4.3 times more active than the TMC-derivative ($IC_{50}$ = 13 nM) and 4300 times more potent than d4T ($IC_{50}$ = 13000 nM).[136]

Similarly, in continued studies, connecting thymidine (THY) and TMC-derivative through a polymethylene linker (ALK) produced a novel bifunctional inhibitor. In particular, compounds THYHP-ALK-TMC (**56**) and THYTP-ALK-TMC (**57**) exhibited higher enzymatic inhibition activity ($IC_{50}$ = 4.3 and 6.0 nM, respectively), with approximately 3- and 2-fold improvement compared to the parent TMC-derivative ($IC_{50}$ = 13 nM). Furthermore, compounds THY-ALK-TMC (**58**) and **56** were effective against the WT strain with $EC_{50}$ values of 0.12 and 0.22 μM, respectively, being 30- and 16-fold superior to that of d4T ($EC_{50}$ = 3.6 μM). Compounds **58** ($CC_{50}$ = 2.2 μM) and **56** ($CC_{50}$ = 6 μM) showed moderate cytotoxicity equivalent to those of TMC-derivative ($CC_{50}$ = 1.9 μM) and ddC ($CC_{50}$ = 4 μM).[137]

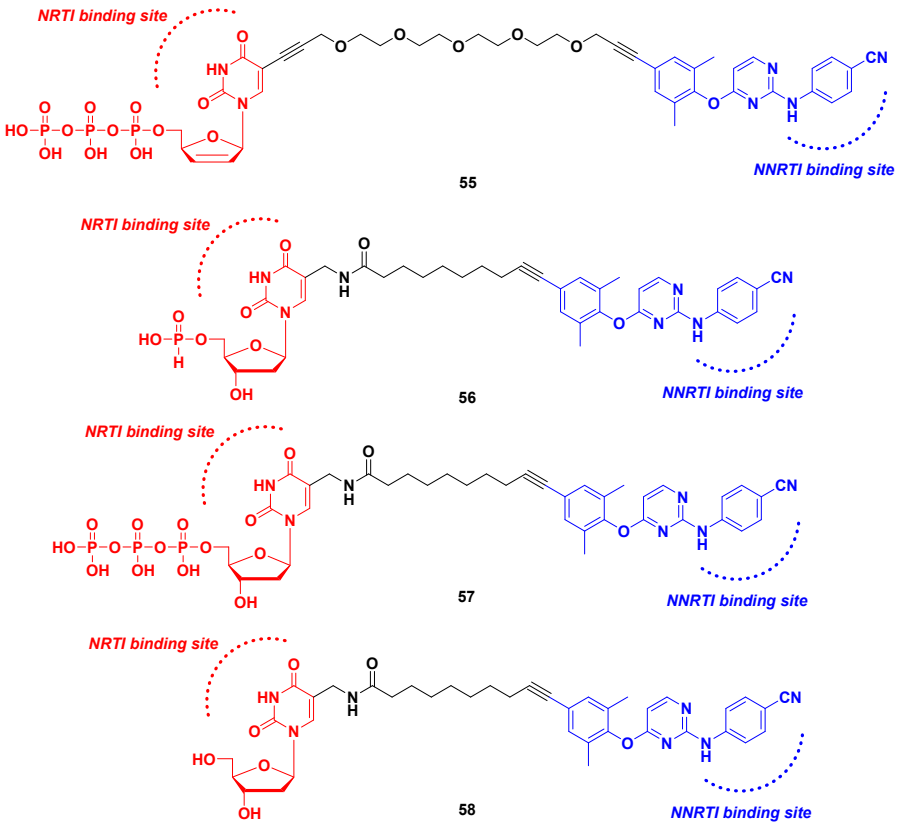


**Figure 26.** Discovery of novel chimeric NRTI/NNRTI bifunctional inhibitors.

To sum up, these encouraging results paved the way for the establishment of proof-of-concept and the design of highly effective bifunctional molecules. Since intracellular phosphorylation is indispensable, future research directions may include embedding bifunctional inhibitors in biodegradable nanogels for drug delivery or using alternative nucleotide-competing RT inhibitors (NcRTIs) that do not require activation by cellular kinases.

## 5. MEDICINAL CHEMISTRY STRATEGIES TO IMPROVE AQUEOUS SOLUBILITY

Drug solubility is a crucial physicochemical property required to characterize the active pharmaceutical ingredient during formulation selection and drug delivery.[138, 139]

Because frequent daily medication remains an intractable issue in the clinical application of NNRTIs due to poor aqueous solubility, there is still a need to search for novel structural analogs that can achieve improved solubility without sacrificing the original efficacy.

**5.1. Prodrug Approach**

The prodrug strategy proved to be an expedient approach to balance both potency and drug-like profiles.[140, 141] Notably, the propionylation modification of the sulfonamide group was effectively confirmed as a potential prodrug strategy, such as the COX-2 inhibitors valdecoxib and parecoxib.[142] GW678248 (**27**), as a novel benzophenone NNRTI, exhibits potent anti-HIV-1 activities toward mutant strains associated with NVP and EFV (**Figure 27**).[79] Its *N*-acylated prodrug form designated GW695634 (**59**) was developed as a potential drug candidate with significantly improved aqueous solubility (Sol. = 92 mg/mL) compared to GW678248 (Sol. = 0.18 μg/mL).[143] GW695634 can be converted into the active metabolite GW678248 by endogenous proteinases.[79, 144] However, GW695634 did not achieve the expected favorable outcome after the phase II trial. In addition, drug-related adverse events including rash, nausea, and diarrhea, were observed in patients. Therefore, by January 2006, the development of GW695634 was discontinued due to safety issues.[145]

In addition, the *N*-propionyl sulfonamide prodrug strategy was successfully applied in the development of elsulfavirine (**61**), a prodrug that received approval in Russia to treat HIV infection and was generated by propionylation of the small molecule selective NNRTI NNRTI VM-1500A (**60**) terminal sulfonamide group

(**Figure 27**).[146] The orally formulated prodrug elsulfavirine can be transformed into the active parent drug VM-1500A through hydrolysis and loss of propionic acid. Elsulfavirine showed excellent tolerability, a favorable safety profile, and high efficacy. The recommended combination regimen includes elsulfavirine, plus two NRTIs, tenofovir disoproxil fumarate and emtricitabine, which was shown to be equally efficacious to EFV-based therapy with fewer adverse CNS and skin adverse events.[147-149]

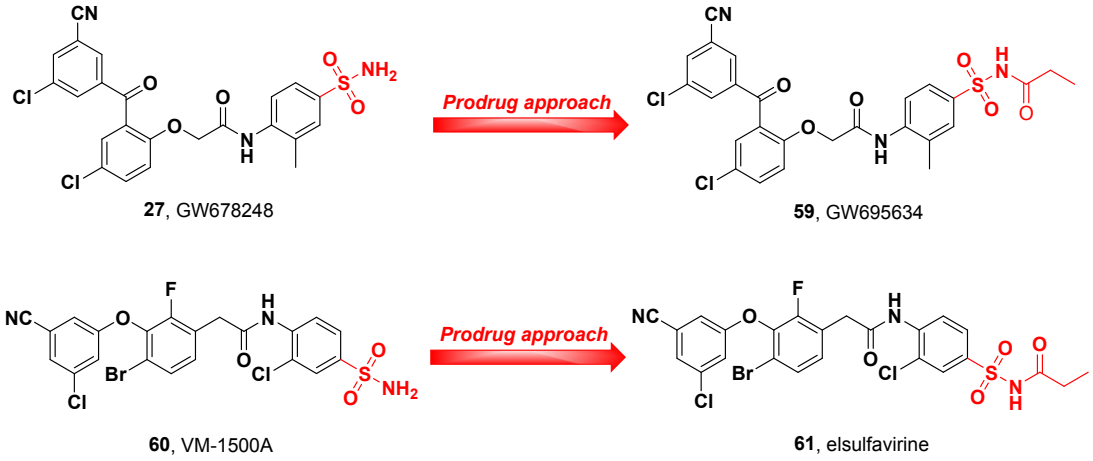


**Figure 27.** Application of the prodrug strategy in GW695634 and elsulfavirine.

By utilizing the prodrug strategy, the replacement of the methylene phosphate group with the *NH* of the DOR triazole ring yielded prodrug **62** with acceptable potency in antiviral and RT inhibitory assays. The aqueous solubility of **62** was 4560 μg/mL, while that of DOR was only 19 μg/mL under the same conditions of pH 7.0. Compound **62** also displayed sufficient chemical stability *in vitro*. All these specific examples suggest the availability and versatility of the prodrug strategy to increase the aqueous solubility of insoluble compounds (**Figure 28**).[150]

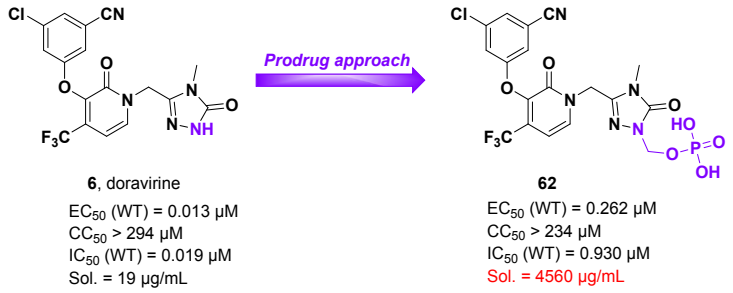


**Figure 28.** Optimization of doravirine *via* a prodrug approach.

## 5.2. Introducing Hydrophilic Groups into the Solvent-Exposed Region

Solvent-exposed regions, as broad modification spaces, are expected to effectively accommodate different kinds of chemical structures and participate in the formation of additional interactions.[151] In addition to the commonly used prodrug strategies, the introduction of solubilizing substituents into the solvent-exposed region has been proven to be equally effective for improving solubility-limited physicochemical properties.

To enhance the antiviral potency of the previously discovered morpholine-containing compound **63** and maintain its good solubility, the introduction of a hydrophilic methylsulfonyl-substituted piperazine moiety yielded compound **64** with moderate activities and improved solubility (**Figure 29**).[152] Thereafter, with a focus on the substituted piperazine fragment pointing toward the solvent-exposed region, the incorporation of dominant scaffolds of **19** and **38** led to the discovery of compounds **65** and **66**, featuring the same sulfonamide group with substantially increased solubility and without severe loss of potency.[153] Currently, the fraction of sp3 carbon atoms is introduced as a parameter to determine the saturation and complexity of spatial structures.[154, 155] Guided by this design assumption, different saturated polar groups were introduced to enrich the SARs around the solvent-exposed region, leading to the

identification of compound **67**, which displayed improved drug resistance profiles against several single mutant strains and favorable water solubility (**Table 7**).[156]

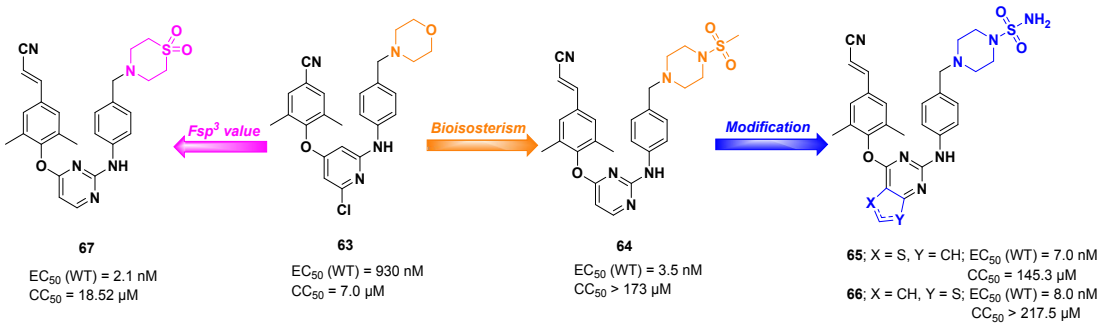


**Figure 29.** Improve the aqueous solubility by introducing hydrophilic substituents into the solvent-exposed region.

**Table 7.** Aqueous Solubility of Compounds **64−67**.

| Compd | 64 | 65 | 66 | 67 | ETV[153] |
|---|---|---|---|---|---|
| **pH = 7.4 (μg/mL)**[a] | - | 39.5 | 38.9 | - | <1 |
| **pH = 7.0 (μg/mL)**[a] | 30.92 | 49.7 | 49.3 | 34.0 | <1 |
| **pH = 2.0 (μg/mL)**[a] | - | 73.1 | 64.9 | - | 127 |

[a]Aqueous solubility: The aqueous solubility of the test compound was determined under different pH values. The assay was measured at least in duplicate.

## 6. MULTITARGET-DIRECTED LIGANDS

In the last few decades, the "one target, one compound" paradigm has achieved unprecedented success in the pharmaceutical industry. However, with the advancement of the pathogenesis of multifactorial disease research, it is evident that this single-target drug does not always guarantee satisfactory efficacy. Although the mixed dose of multiple drugs featuring complementary mechanisms of action may be a quicker and more flexible approach for multitarget therapeutics, such combination is often hindered

by poor patient compliance and unpredictable PK profiles.[157, 158] An alternative approach, termed the multitarget-directed ligand (MTDL) strategy, considers developing drugs that can interact with more than one biological target involved in complex diseases.[159] Currently, the rational design of multifunctional ligands by subtly combining the essential pharmacophores of multiple drugs acting on various targets is attracting significant attention.[160] The concept of the MTDL strategy is particularly applicable to HIV-1 inhibitor design, with the apparent virtue of simultaneously modulating multiple targets during the virus replication process.

Integrase (IN) is responsible for catalyzing the essential process of viral DNA integration into host DNA. Since IN has no homologous enzyme in humans, both IN and RT are regarded as validated targets for developing anti-HIV drugs. Moreover, the high structural and functional analogies between RT and IN led to the similarity of the pharmacophores required by inhibitors, providing the foundation for the intentional design of dual inhibitors.[161, 162] Introducing the aryl diketoacid moiety of IN inhibitor **68** at the *N*-1 position of HEPT-type NNRTI TNK-651 (**15**) afforded four hybrid inhibitors (**Figure 30**). The enzyme inhibition assay results showed that the activity of all the dual inhibitors was greater than or similar to that of **15** against RT, indicating that the open channel surrounding the *N*-1 phenyl could accommodate structural modifications. All tested compounds also potently inhibited IN at low micromolar concentrations, at least one order of magnitude lower than that of **68**. The best compound **69** displayed excellent potency against HIV-1 ($EC_{50}$ = 9.7 nM) and a high selectivity index (SI > 1000).[163] The discrepancy between the RT and IN inhibitory

activities prompted a detailed exploration of SARs by systematic modification of compound **70**, including the insertion of benzyl into the N-1 linker, the extension of the N-1 linker, and the incorporation of a diketoacid group into the C-2 or N-3 linker. However, four subseries of compounds exhibited weak or lost potency against RT and IN.[164] On the other hand, substituting the diketoacid functionality at the C-5 or C-7 position of the approved drug delavirdine's indole ring to replace the original methyl sulfonamide group yielded a series of delavirdine derivatives. The biological activity results showed that the C-5 substitution was preferred over the C-7 substitution, probably because the angular conformation of the latter might not be conducive for binding to IN and partially reduce the electrostatic interactions with K103 and K104 in RT. It was also observed that substituting a halogen at indole significantly decreased the RT-inhibition potency and slightly increased the IN-inhibition potency, achieving the equilibrium activities toward RT and IN of compounds **71** and **72**.[165]

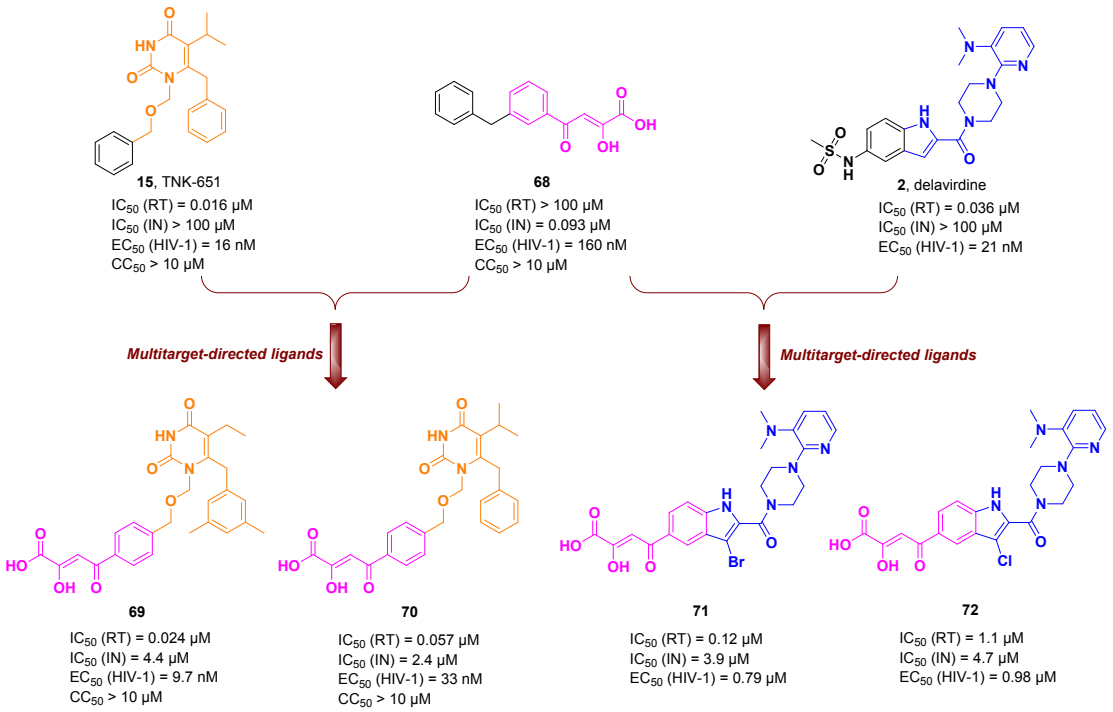

**Figure 30.** Design of multitarget-directed ligands as RT/IN dual inhibitors.

The simplified pharmacophore model revealed that IN binding requires a minimum of two magnesium ions chelating triad and one hydrophobic benzyl group. *N*-3 hydroxylation of the HEPT analog afforded a series of pyrimidine-2,4-dione derivatives. The development of this series was encouraged by molecular docking into NNIBP and the IN catalytic core domain (CCD). The new hydroxy group could maintain the key hydrogen bonding between the ligand and K101 in NNIBP to avoid significantly compromising the affinity with RT. The C(2)O–N(3)OH–C(4)O acts as a functional group to chelate two metal ions and, together with the benzyl moiety, meets the basic pharmacophore requirements. SAR analysis indicated that the OH group at *N*-3 was indispensable for the inhibitory effect on IN and that the oxybenzyl moiety at *N*-1 was also important for IN inhibition (**Figure 31**). The most potent compound **74** was able to dually inhibit IN and RT, and **74** also displayed equipotent antiviral potency compared to TNK-651 ($EC_{50}$ = 13 nM).[166] After the discovery of potent dual inhibitor **74** with a novel chemotype for an IN inhibitor, further optimizations focused on the three structural features around lead **74**, namely, the aromatic and chelating domains, linker domain, and hydrophobic domain. The SARs results can be summarized as follows: further improvement in anti-IN activity can be achieved by halogen substitution of the *N*-1 benzyl under the premise of keeping the *N*-3 OH group unchanged. The length and atomic nature of the linker affect the affinity of the molecule to IN by controlling the spatial orientation between the chelating functionality and *N*-1 oxybenzyl moiety, and the optimal linker should include a carbon chain of 3 to 4 atoms.

The hydrophobic domain has an elusive influence on IN inhibition, and this region predominantly affects the activity in cooperation with the linker domain. This study resulted in the discovery of compounds **73**−**75**, featuring a $CH_2OCH_2$ linker offering both high IN-inhibitory activity and favorable anti-HIV potency for further development.[167]

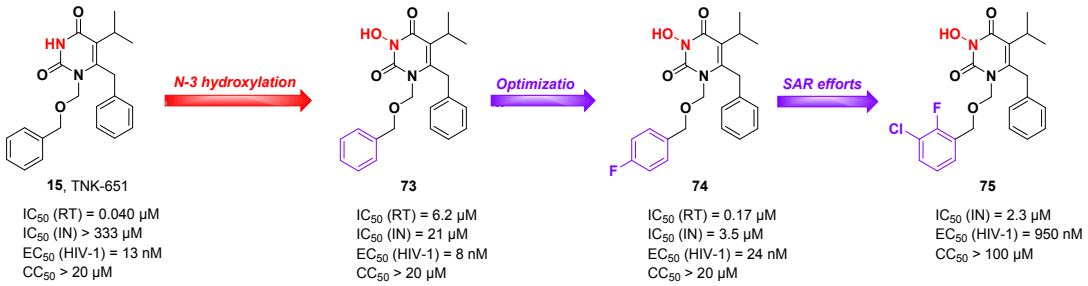


**Figure 31.** Optimization of TNK-651 as an RT/IN dual inhibitor.

## 7. CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Since the first clinical identification of AIDS in the 1980s, significant scientific progress has been made in exploring related therapeutic treatments. However, the growing number of HIV-infected people reminds us that AIDS continues to be a severe pandemic disease throughout the world, and unremitting efforts are still needed to develop novel anti-HIV agents due to the lack of vaccines.

Drug discovery is a complex, expensive, and lengthy endeavor that includes a range of procedures from hit compound discovery to preclinical evaluation to clinical verification, especially when searching for pioneering drugs that target novel and unproven therapeutically relevant mechanisms. The elusiveness of therapeutically beneficial targets has prompted medicinal chemists to exploit validated targets thoroughly. In contrast, follow-up campaigns seem to be more economical and safer for

generating potential chemical entities with clinical application differentiation by optimizing known bioactive molecules. Therefore, due to its unique mechanism of action and well-known 3D structure, RT has attracted much attention as a validated target in drug design and spawned potent NNRTIs as a major component of the HAART regimen.

Significant progress has been achieved in the lead discovery and lead optimization of different classes of NNRTIs by applying classical medicinal chemistry strategies, as reviewed above. However, despite the success of many approved NNRTIs, drug resistance still cannot be prevented due to the evolution of HIV-1 strains, especially under drug selective pressure, and key amino acid mutations in NNIBP may greatly affect the binding affinity between inhibitors and RT. In addition, there is little evidence that any mutation can reduce the sensitivity to only one drug, so almost all mutations confer a high level of cross-resistance to NNRTIs. Notably, precise sequence detection of mutated amino acids has proven to be a prerequisite for successfully overcoming drug resistance. This is an arduous multidisciplinary task, and it is reasonable to cooperate closely with structural biologists while applying traditional medicinal chemistry as the core component.

Currently, a new technology termed proteolysis targeting chimera (PROTAC) takes advantage of the intracellular mechanism of the ubiquitin–proteasome system to specifically degrade and eliminate proteins related to human diseases by simultaneously binding the target of interest and the E3 ubiquitin ligase.[168, 169] The PROTAC strategy has been successfully used to identify effective molecules that inhibit and induce

hepatitis C virus (HCV) protease degradation.[170] However, it is now clear that HIV-1 reverse transcription takes place within the intact capsid in infected cells.[171, 172] The capsid plays important roles in the virus replication cycle, maintaining RT and genetic material in the internal environment and thereby increasing the efficiency of reverse transcription. Thus, we wish to point out that RT will not be an accessible target for ubiquitination or degradation using a PROTAC strategy, as the E3 ligase ligand moiety of the PROTAC molecule cannot normally recruit the E3 ubiquitin ligase in the host cell to induce ubiquitination and degradation.

As diverse modern drug discovery strategies continue to mature and improve, the development of novel NNRTIs will move from fortuitous discovery and trial-and-error approaches to an elaborate design. In this regard, we discussed potential design strategies for the rational optimization of new generation NNRTIs from the perspective of medicinal chemistry.

**7.1. Advantages of Conformational Flexibility**

Resistance to first-generation drugs has developed rapidly because the structural rigidity of these drugs prevents them from adapting to amino acid mutations. In contrast, the intrinsic features of second-generation NNRTIs ETV and RPV with torsional flexibility ("wiggling") and repositioning ability ("jiggling") allow them to adjust to changes in the mutated NNIBP and minimize the loss of binding stabilization.[109, 111] ETV and RPV, with a more flexible horseshoe conformation, can be accommodated well within variational NNIBP based on molecular movements and tiny side chain rearrangements. More importantly, crystallographic studies revealed that RPV can

induce localized changes in NNIBP by using the structural flexibility of RT, thereby establishing interactions with Y183 to compensate for the weakened π–π stacking interactions after Y181C substitution.[112] It is evident that conformational flexibility and positional adaptability ensure efficient binding and account for their maintained potency in the presence of resistant mutations. Therefore, the exploitation of more flexible inhibitors while taking advantage of the potential flexibility of RT within a reasonable range raises a new opportunity to overcome drug resistance by adopting multiple conformations to adapt for the mutated binding pockets.

### 7.2. Targeting Highly Conserved Residues

Earlier-generation NNRTIs mainly relied on the π–π stacking interaction with aromatic residues Y181 and Y188, which were lost when tyrosine was replaced by non-aromatic cysteine or leucine, resulting in drug resistance to these inhibitors.[30, 31] The design of novel NNRTIs should minimize the dependence on the interactions with the readily mutated residues Y181 and Y188 while strengthening the interaction with the highly conserved region composed of F227, W229, and L234, which are less prone to mutation. Analysis of the mutation nature of residues and identification of highly conserved residues within NNIBP are essential for rational design.

### 7.3. Developing Main Chain Hydrogen Bonds

The pyrimidinyl nitrogen and the right imino linker of ETV and RPV form the "signature" dual hydrogen bonds with the K101 backbone that are necessary to maintain binding affinity.[173] In addition, the left imino linker of RPV participates in hydrogen-bonding interactions with the E138 main chain *via* a bridging water molecule.

Similarly, it can be observed that DOR is involved in a key hydrogen bond between the methyltriazolone moiety and the K103 backbone.[87] The extensive hydrogen-bonding network between ligands and the amino acid main chain greatly contributes to the free energy for target binding, and it will continue to be maintained regardless of the side chain mutations.

### 7.4. Targeting Solvent-Exposed Regions

The solvent-exposed region provides potential chemical space for substantial modifications of known bioactive compounds, commonly by introducing charged and polar groups to enhance binding affinity with the protein or introducing additional pharmacophores to construct multifunctional ligands. Consistently, NNRTIs are mostly hydrophobic, so the introduction of hydrophilic groups can also improve the pharmacokinetic profiles for oral administration.

### 7.5. Designing Covalent Inhibitors

The covalent drug design strategy offers potential gains such as higher potency, reduced drug resistance, and prolonged duration of target engagement. In addition to the common acrylamide and sulfonyl fluoride that have been successfully applied, boric and boronic acids are able to be used as functional groups in the construction of covalent inhibitors.[174] The distinction between boron atoms and carbon atoms lies in the presence of an empty p-type atomic orbital that allows the reversible formation of covalent bonds with oxygen nucleophilic residues. The development of specific warheads chemically reactive to certain residues is expected to provide new covalent NNRTIs with high activity and few off-target effects. In addition to the rational selection of warheads to

modulate optimal covalent reactions, continuous attempts should be made to enhance non-covalent and specific binding to target proteins reversibly.

### 7.6. Introducing Privileged Halogen Atoms

The optimization of EFV suggested that the trifluoromethyl group can reduce the molecular pKa value and increase the affinity with NNIBP through strong hydrogen-bonding interactions.[175] Furthermore, the introduction of trifluoromethyl to the pyridone scaffold of DOR led to improved plasma stability and a prolonged half-life.[87] In addition to fluorine, the bromine atom of ETV contributes to the formation of electrostatic interactions with L100 and V179 to enhance antiviral activity.[109] As privileged substituents in NNRTI design, halogen atoms play an important role in improving metabolic stability and optimizing pharmacological parameters such as lipophilicity and permeability. In addition, the steric effects of halogens allow these bulky atoms to fully occupy the chemical space of the target. More recently, halogen bonds involving an electrophilic region of the halogen atom with a nucleophilic region of the halogen-bonding acceptor have been recognized to be beneficial for the stability of molecule–protein interactions.

### 7.7. Exploiting Unconventional Binding Sites

The discovery of NNRTIs acting at newly emerging binding sites contributes to the exploration of potential interactions and enhancement of binding affinity to the target as much as possible. Fragment screening based on crystallographic studies identified seven novel binding sites of RT, three of which (knuckles, NNRTI-adjacent, and incoming nucleotide binding) proved to be inhibitory in enzymatic assays.[133]

Further crystallographic studies of 2,4,5-trisubstituted pyrimidines revealed that the pyridine ring extends from the NNIBP toward the NNRTI-adjacent site, allowing the design of dual-site NNRTIs through fragment linking or merging.[66] Moreover, the proximity of the NRTI-binding pocket and the NNRTI-binding pocket enabled the simultaneous occupation of two sites to achieve synergistic inhibition of RT. Very recently, cocrystal structures of RT and gp120 antagonists indicated that they can inhibit RT by linking the NRTI-binding pocket and the NNRTI-binding pocket.[176] Notably, the rational design of bifunctional NNRTIs as an alternative strategy to enhance activity and alleviate drug resistance needs to take into account the simplification of individual pharmacophores, the positions of attachment points on multiple ligands, and the nature and length of the specific linker that orient the ligands in an appropriate binding conformation.

**7.8. Targeting Host Factors involved in Reverse Transcription**

In addition to the interactions between viral proteins and ligands, host factors closely related to pathogenic targets need to be considered seriously.[177] Compared to drugs targeting viral proteins, host-targeting antiviral agents possess broad-spectrum antiviral activities and higher genetic barriers, providing new insights for overcoming drug resistance.[178] Host-derived RNA helicase A (RHA) is involved in many key stages of the viral replication process and can be assembled together with the viral RNA genome into the capsid core.[179, 180] RHA, as an RT processivity factor, promotes reverse transcription efficiency, so viruses lacking RHA are less infectious due to the reduced DNA synthesis rate generated from genomic RNA.[181] Since viruses are less likely to

mutate to replace missing host cell functions needed for viral replication, the possibility of selecting resistant mutants toward host-directed agents is reduced. Nonetheless, continued application of host-targeting drugs may facilitate viral switching to bind alternative host factors or enhance viral efficiency to replicate optimally under limited host factors; for example, emerged strains with partial maraviroc resistance may use alternate co-receptors CXCR4 or alter its binding affinity with CCR5.[182-184] Therefore, the key to developing antiviral drugs that target host factors (such as RHA) involved in reverse transcription is to clearly elucidate the internal connection and mechanism of action between the host cell and HIV-1.

**7.9. Long-Acting Formulations and Preexposure Prophylaxis Agents**

Poor compliance leads to irregular drug concentrations and incomplete viral suppression that in turn readily induces drug resistance and treatment failure. Consequently, the application of alternative long-acting formulations should be conducive to overcoming patient compliance challenges that lead to drug resistance. The new long-acting regimen containing extended release injectable nanosuspensions of cabotegravir plus rilpivirine was approved by the U.S. FDA in 2021 as the first injectable complete regimen (Cabenuva®) due to its good tolerance, lack of adverse reactions, and non-inferiority to the current standard oral therapy.[185-187]

In particular, we wish to emphasize that the fight against AIDS is a long-term campaign, so the use of PrEP agents in high-risk individuals and the popularization of related prevention knowledge are also particularly important. Another promising DAPY-type NNRTI dapivirine, which was developed in the form of a vaginal

microbicide, was approved by the European Medicines Agency (EMA) in 2020 as an effective PrEP agent for HIV-1 prevention.[188-190] As prescriptions and treatments become more personalized, the application of precision medicine based on individual patient information may be promising in the future, despite being relatively advanced in the field of antiviral therapy.

**7.10. Prediction and Evaluation of ADMET Properties**

Finally, most promising drug candidates are eventually forced to be suspended at the late stage of drug development due to their toxicity or poor PK profiles rather than efficacy. For example, fosdevirine (GSK2248761) is a potent and selective NNRTI drug candidate previously developed by the GSK team.[191] However, the high incidence of delayed seizures after administration and persistent seizures after withdrawal observed in treatment-experienced individuals ultimately led to the failure of its development after a phase IIb clinical trial.[192] The predominant cysteine-conjugated metabolite derived from the Michael addition of the electrophilic cyanovinylphenyl group of fosdevirine with glutathione was considered to be the cause of CNS toxicity.[126] In addition, animal toxicity, a high oxidative metabolism rate, and poor effects of the combination therapy caused the development of capravirine (S-1153, AG1549) to be suspended after phase IIb clinical trials.[193, 194] Therefore, in the optimization process of NNRTIs, more attention should be given to early drug-likeness research, especially the prediction and evaluation of ADMET properties.

However, the testing capabilities of *in vivo* and *in vitro* ADMET assay seem stretched thin in the face of large amounts of screening efforts. Currently, *in silico*

ADMET prediction platforms have been developed to evaluate these properties in an economical and efficient way. Available open-access tools such as admetSAR 2.0[195] and ADMETlab 2.0[196] are fully integrated with ADMET prediction platforms that are capable of excluding undesirable drug candidates, which can predict ADMET from their chemical structure before practical synthesis. The accuracy of an *in silico* ADMET prediction tool depends on the quality of the experimental data in the database, the validation criteria and the design ideas of the developed model. To obtain reliable predictions, researchers should use different *in silico* tools for comparison, followed by identifying the most likely prediction results.

Currently, the development of antiviral drugs with low toxicity and high tolerability to improve patient compliance remains a challenging task. Therefore, the application of a drug repositioning strategy to screen anti-HIV hits from available drugs with reliable physicochemical properties and safety will also demonstrate great potential, which has been proven to be valuable and powerful in the prompt treatment of the recent large-scale outbreak of the infectious virus SARS-CoV-2.[197, 198]

In summary, this article describes the molecular structure and discovery campaign of novel representative NNRTIs while elaborating significant bioactive compound optimization strategies associated with the solution of intractable scientific issues. Overall, there is still a continuing demand for synergistic collaboration between medicinal chemistry strategies, crystallographic studies, and computer-aided tools to achieve the ultimate goal of obtaining new NNRTIs with improved genetic barriers, desirable pharmacokinetic profiles, and excellent safety properties.

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## ABBREVIATIONS

AIDS, acquired immune deficiency syndrome; $CC_{50}$, 50% cytotoxicity concentration; CCD, catalytic core domain; CL, clearance rate; CuAAC, copper(I)-catalyzed azide-alkyne cycloaddition; CYP, cytochrome P450; d4T, 2′,3′-didehydrothymidine; DAPY, diarylpyrimidine; DLV, delavirdine; DOR, doravirine; $EC_{50}$, 50% effective concentration; EFV, efavirenz; ETV, etravirine; FDA, Food and Drug Administration; FEP, free energy perturbation; HAART, highly active antiretroviral therapy; HIV-1, human immunodeficiency virus-1; $IC_{50}$, 50% inhibition concentration; IN, integrase; MTDL, multitarget-directed ligand; NcRTIs, nucleotide-competing reverse transcriptase inhibitors; NNIBP, NNRTI-binding pocket; NNRTIs, nonnucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; NVP, nevirapine; PDB, protein data bank; PK, pharmacokinetics; PrEP, preexposure prophylaxis; RPV, rilpivirine; RT, reverse transcriptase; SARs, structure–activity relationships; $T_{1/2}$, half-life; UNAIDS, the Joint United Nations Programme on

HIV/AIDS; WT, wild-type.

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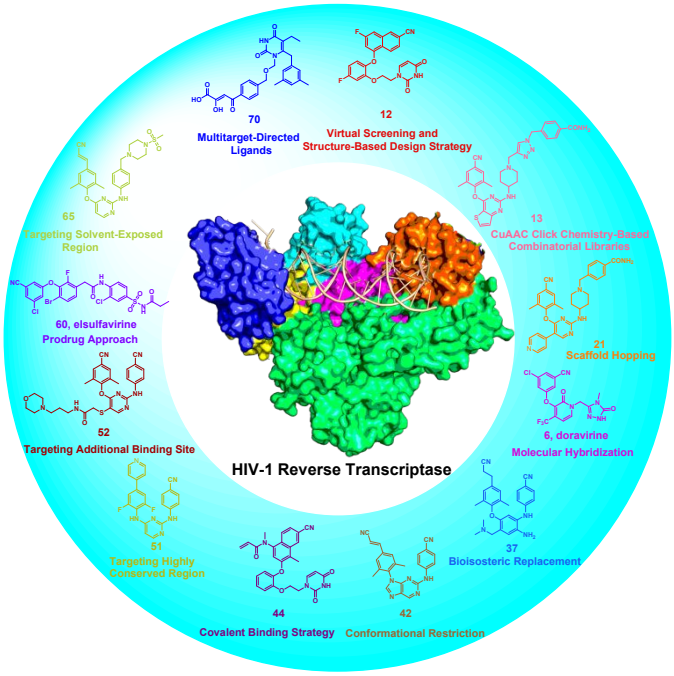
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