Further Exploring Solvent-Exposed Tolerant Regions of Allosteric Binding Pocket for Novel HIV-1 NNRTIs Discovery

Dongwei Kang,[†] Zhao Wang,[†] Heng Zhang,[†] Gaochan Wu,[†] Tong Zhao,[†] Zhongxia Zhou,[†] Zhipeng Huo,[†] Boshi Huang,[†] Da Feng,[†] Xiao Ding,[†] Jian Zhang,[†] Xiaofang Zuo,[†] Lanlan Jing,[†] Wei Luo,[†] Samuel Guma,[‡] Dirk Daelemans,[‡] Erik De Clercq,[‡] Christophe Pannecouque,[‡] Peng Zhan,^{*,†} and Xinyong Liu^{*,†}

[†]Department of Medicinal Chemistry, Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Shandong University, 44 West Culture Road, 250012 Jinan, Shandong, P. R. China

[‡]Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, K.U. Leuven, Herestraat 49 Postbus 1043 (09.A097), B-3000 Leuven, Belgium

S Supporting Information

ABSTRACT: Based on the detailed analysis of the binding mode of diarylpyrimidines (DAPYs) with HIV-1 RT, we designed several subseries of novel NNRTIs, with the aim to probe biologically relevant chemical space of solvent-exposed tolerant regions in NNRTIs binding pocket (NNIBP). The most potent compound **21a** exhibited significant activity against the whole viral panel, being about 1.5–2.6-fold (WT, EC₅₀ = 2.44 nM; L100I, EC₅₀ = 4.24 nM; Y181C, EC₅₀ = 4.80 nM; F227L + V106A, EC₅₀ = 17.8 nM) and 4–5-fold (K103N, EC₅₀ = 1.03 nM; Y188L, EC₅₀ = 7.16 nM; E138K, EC₅₀ = 3.95 nM) more potent than the reference drug ETV. Furthermore, molecular simulation was conducted to understand the binding mode of interactions of these novel NNRTIs and to provide insights for the next optimization studies.

KEYWORDS: HIV-1, NNRTIs, DAPY, tolerant regions, drug design

Human immunodeficiency virus (HIV) is the cause of the acquired immunodeficiency syndrome (AIDS), which still remains a major threat for human health and life since it was first discovered in 1981.¹ Recent scientific evidence has demonstrated that highly active antiretroviral therapy (HAART) regimens are the most effective treatment regimen for HIV-1 infection.^{2–4} The non-nucleoside reverse transcriptase inhibitors (NNRTIs) remaining one of the key components of HAART for their potent antiviral activity, high selectivity, and favorable pharmacokinetics.^{5,6}

The NNRTIs bind with an allosteric site (non-nucleoside inhibitor binding pocket, NNIBP) in HIV-1 RT in a noncompetitive manner and then induce conformational changes of the catalytic domain and lead to inhibit its DNA polymerase activity.⁷ Nevirapine (NVP), delavirdine (DLV), and efavirenz (EFV) (Figure 1) are first-generation NNRTIs for AIDS therapy.⁸ A major concern of them is that the rapid

development of drug resistance reduced their effectiveness. Especially, K103N and Y181C are the most prevalent mutations in clinical NNRTI-resistant HIV-1 isolates.⁹ The second-generation agents, etravirine (ETV) and rilpivirine (RPV), exhibit a broad spectrum of activity against clinically relevant HIV-1 mutant strains and enjoyed considerable clinical success.¹⁰ Although ETV and RPV display higher genetic barriers to emergence of drug resistance, mutant strains with reduced susceptibility to ETV and RPV have emerged rapidly.⁹ In addition, adverse effects such as hepatotoxicity, severe rash, and central nervous system side effects continue to emerge in patients receiving HAART.^{11,12} These effects have led

Received: February 2, 2018

Accepted: March 1, 2018

Published: March 1, 2018

Figure 1. Marketed HIV NNRTIs and thiophene[3,2-*d*]pyrimidine lead compounds 6a and 6b (K-5a2 and 25a).

Figure 2. Strategy for further structural optimization of K-5a2.

medicinal chemists to develop novel antiretroviral agents with improved resistance and drug-like profiles.

Structure-based design is typically focused on the optimization of a chemical series to increase affinity toward a mutable target protein, to probe biologically relevant chemical space, and to improve the drug-like profiles. Under this situation, recently, to discover structurally different and best-in-class NNRTIs, our group explored multiple series of NNRTIs and finally identified the candidates K-5a2 and 25a with significantly improved drug resistance profiles.^{13–18}

Being inspired by this, subsequent structural optimization of K-5a2 was conducted to explore the left wing, central ring, tolerant region I, and tolerant region II, employed by structural bioinformatics and bioisosterism drug design strategy (Figure 2). Based on the known SARs, we decided to incorporate five- or six-membered heterocycles at the tolerant region I first, and hope to develop new hydrogen bond interactions with amino acid residues of the NNIBP and to improve drug resistance profiles. Then the left wing was explored by connecting aromatic ring to the left wing via an alkynyl chain with the aim to maintain the additional interactions with highly conserved

residues Phe227 and Trp229. Finally, as a matter of experience, a potential strategy to overcome drug resistance is the rational design of a novel inhibitor with a different chemical scaffold. Even though we have investigated thiophene[3,2-*d*]pyrimidine derivatives as potent NNRTIs, we recognized that the center core represented an area that had remained largely unexplored. Thus, the privileged structure pyrrolo[3,2-*d*]pyrimidine was employed to replace the thiophene[3,2-*d*]pyrimidine central ring. Meanwhile, to further explore the solvent-exposed tolerant region II, the substituent groups with different configurations were installed to the N atom of the pyrrolo[3,2-*d*]pyrimidine, anticipating that they might interact favorably with this region.

The synthetic protocols for the newly designed compounds are outlined in Schemes S1–3. All the derivatives were fully characterized by high resolution mass spectrum (HRMS), proton nuclear magnetic resonance (¹H NMR) spectroscopy, and carbon nuclear magnetic resonance (¹³C NMR) spectroscopy (see Supporting Information).

Antiviral potency was evaluated with MTT method using MT-4 cell cultures infected with WT HIV-1 strain (IIIB) as well as cells infected with a panel of NNRTI-resistant single- and

Table 1. Structures, Anti-HIV-1 Activity, Cytotoxicity, and Inhibitory Activity against HIV-1 RT of 9a–9f

compd	EC ₅₀ (nM) ^a		CC ₅₀ (μM) ^b	SI ^c		IC ₅₀ (μM) ^d
	IIIB	RES056		IIIB	RES056	
9a	13.7 ± 11.1	17058 ± 6063	>254	>18473	>15	0.055
9b	3.55 ± 1.48	>271993	>254	>76531	X1	0.082 ± 0.006
9c	5.49 ± 3.67	1168 ± 91.7	24.3 ± 3.42	4425	21	0.052 ± 0.003
9d	5.67 ± 1.53	2703 ± 44.1	21.1 ± 2.92	3722	8	0.053 ± 0.002
9e	7.19 ± 3.16	8970	133 ± 7.07	18607	15	0.022 ± 0.004
9f	32.8 ± 23.3	>4362	4.35 ± 0.266	133	<1	0.221 ± 0.032
EFV	2.85 ± 1.34	224 ± 114	>6.336	>2223	>28.2	0.006 ± 0.001
NVP	124 ± 8.40		>15.020	>121	X1	0.879 ± 0.298
ETV	4.47 ± 0.162	34.4 ± 13.7	2.155 ± 0.107	482	62.6	
K-5a2	1.43 ± 0.43	30.6 ± 12.5	>227	>159101	>7438	

^aEC₅₀: concentration of compound required to achieve 50% protection of MT-4 cell cultures against HIV-1-induced cytotoxicity, as determined by the MTT method. ^bCC₅₀: concentration required to reduce the viability of mock-infected cell cultures by 50%, as determined by the MTT method. ^cSI: selectivity index (CC₅₀/EC₅₀). ^dIC₅₀: inhibitory concentration of test compound required to inhibit biotin deoxyuridine triphosphate (biotin-dUTP) incorporation into WT HIV-1 RT by 50%.

Table 2. Structures, Anti-HIV-1 Activity, and Inhibitory Activity against HIV-1 RT of 14a–14c

compd	R ²	EC ₅₀ (nM)		CC ₅₀ (μM)	SI		IC ₅₀ (μM)
		IIIB	RES056		IIIB	RES056	
14a	pyridine-4-yl	33.8 ± 21.5	223 ± 28	11.51 ± 3.74	340	52	0.86 ± 0.07
14b	pyridine-3-yl	40.8 ± 4.67	153 ± 6.0	6.39 ± 4.12	157	42	0.28 ± 0.01
14c	pyridine-2-yl	9.0 ± 2.7	888 ± 201	10.27 ± 8.28	1130	12	0.29 ± 0.10
NVP		281 ± 38	>15020	>15.02	>53	X1	0.59 ± 0.15
ETV		4.3 ± 0.4	46 ± 17	4.59	1050	100	
AZT		7.0 ± 1.0	17 ± 6.0	>7.48	>1004	>448	
K-5a2		1.43 ± 0.43	30.6 ± 12.5	>227	>159101	>7438	
25a		1.2 ± 0.2	5.0 ± 0.8	2.30 ± 0.46	1882	419	0.16

double mutant strains, such as L100I, K103N, Y181C, Y188L, E138K, F227L + V106A, and K103N + Y181C (RES056). Nevirapine (NVP), efavirenz (EFV), and zidovudine (AZT) were selected as control drugs. The values of EC₅₀ (anti-HIV potency), CC₅₀ (cytotoxicity), SI (selectivity index, CC₅₀/EC₅₀ ratio), and IC₅₀ (enzyme inhibition activity) of the synthesized compounds are summarized in Tables 1–3 and S1–S2.

As expected, when we conducted structural optimization of tolerant region I, most of the newly synthesized compounds (9a–e) exhibited significant antiviral potency against WT HIV-1 with low nanomolar EC₅₀ values of 3.55–13.7 nM, only compound 9f with a phenylpyrrolidine substituent displayed decreased potency (EC₅₀ = 32.8 nM), which supported our previous hypothesis that hydrogen bond donor and receptor groups in the solvent-exposed tolerant region I play an

important role to enhance antiviral activity. Notably, compound 9b (EC₅₀ = 3.55 nM, SI > 76531), 9c (EC₅₀ = 5.49 nM, SI = 4425), and 9d (EC₅₀ = 5.67 nM, SI = 3722) were identified as the most potent inhibitors against WT HIV-1, being more potent than NVP (EC₅₀ = 124 nM, SI > 121) and as potent as the reference drug ETV (EC₅₀ = 4.47 nM, SI = 482). Elaboration with a sulfone methyl group at the N₄-position in imidazole of 9c yielded 9d with equivalent activity. Additionally, compounds 9a and 9e turned out to be potent inhibitors with EC₅₀ values of 13.7 and 7.19 nM, which was slightly inferior to that of the reference drug ETV. However, all derivatives were weakly effective against double mutant strain RES056. Furthermore, it is noteworthy that all compounds displayed reduced cytotoxicity compared to ETV.

In the case of the mutant HIV-1 strains (Table S1), 9b–9e were also demonstrated with potent activity against single

Table 3. Structures, Anti-HIV-1 Activity, and Inhibitory Activity against HIV-1 RT of 20a–20c, 21a–21c, and 22a–22c

20a-c R⁴ =

21a-c R⁴ = H

22a-c R⁴ =

compd	R ³	EC ₅₀ (nM)		CC ₅₀ (μM)	SI		
		IIIB	RES056		IIIB	RES056	IC ₅₀ (μM)
20a	–SO ₂ NH ₂	8.90 ± 3.36	110 ± 3.68	3.67 ± 0.227	454	33	0.193 ± 0.031
20b	–SO ₂ CH ₃	30.8 ± 21.9	187 ± 12.7	5.15 ± 0.831	270	26	0.380 ± 0.072
20c	–CONH ₂	16.5 ± 13.6	2288 ± 141	>39.3	227	2	0.195 ± 0.035
21a	–SO ₂ NH ₂	2.44 ± 0.344	85.0 ± 8.92	1.91 ± 1.04	1052	61	0.075 ± 0.010
21b	–SO ₂ CH ₃	5.92 ± 1.17	116 ± 6.87	8.12 ± 2.47	589	16	0.098 ± 0.020
21c	–CONH ₂	5.72 ± 2.04	113 ± 14.4	15.9 ± 4.54	1829	118	0.108 ± 0.025
22a	–SO ₂ NH ₂	9.51 ± 3.95	484 ± 8.67	4.84 ± 0.769	632	10	0.163 ± 0.005
22b	–SO ₂ CH ₃	26.3 ± 17.0	547 ± 39.5	10.0 ± 2.83	459	17	0.189 ± 0.038
22c	–CONH ₂	35.7 ± 15.5	567 ± 15.6	6.44 ± 3.62	177	9	0.158 ± 0.005
NVP		281 ± 38	>15020	>15.020	>53	X1	0.879 ± 0.298
ETV		4.0 ± 0.3	35 ± 13	2.155 ± 0.107	541	62	
AZT		7.0 ± 1.0	17 ± 6.0	>7.483	>1004	>448	
K-Sa2		1.43 ± 0.43	30.6 ± 12.5	>227	>159101	>7438	
25a		1.2 ± 0.2	5.0 ± 0.8	2.30 ± 0.46	1882	419	

mutant HIV-1 strains, especially to K103N and E138K. Compounds **9c–9e** turned out to be single-figure-nanomolar inhibitors of K103N with EC₅₀ values of 5.98, 4.46, and 6.13 nM, respectively, which was equivalent to that of ETV (EC₅₀ = 5.39 nM). Furthermore, **9b–9e** inhibited the K103N mutant strain with EC₅₀ values of 8.81, 12.0, 13.4, and 6.95 nM, respectively, being more potent than NVP and ETV. However, all of them are weakly effective against other single and double mutant HIV-1 strains.

In addition, enzymatic assay were performed to validate the binding target of these newly designed derivatives. The results demonstrated these compounds could specifically target HIV-1 RT, and **9a–9e** showed extremely high RT inhibitory activity with IC₅₀ values of 0.022–0.082 μM, with an exception of compound **9f** (IC₅₀ = 0.221 μM). Coincidentally, **9f** exhibited the highest EC₅₀ values compared to **9a–e**. These data indicated that the activity between HIV-1 replication and RT activity appeared to have good consistency. Incorporation of hydrophilic substituents containing hydrogen bond donor and receptor groups in the solvent-exposed tolerant region I was not only beneficial for their anti-HIV-1 activity but also for their inhibitory activity against RT.

Then, we focused our attention on the novel thiophene[3,2-*d*]pyrimidine derivatives (**14a–c**) in which the left wing of K-Sa2 was replaced by ethynylpyridine. All three compounds exhibited nanomolar activity toward WT HIV-1 strain. More importantly, they all have lower cytotoxicity (CC₅₀ = 11.51, 6.39, and 10.27 μM, respectively) than **25a** (CC₅₀ = 2.30 μM), which validates the rationality of our design. Among them, **14c** exhibited the most potent activity against WT HIV-1 (EC₅₀ = 9.0 nM, SI = 1130), it was notably more active than NVP but lower than that of ETV. All these three derivatives were also active at submicromolar concentrations against RES056, and **14b** was the most active inhibitor with EC₅₀ value of 153 nM, which was superior to NVP (EC₅₀ > 15020 nM) but still inferior to ETV (EC₅₀ = 46 nM).

Additionally, **14a–14c** showed potent RT inhibitory activity with IC₅₀ value of 0.86, 0.28, and 0.29 μM, being superior or comparable to NVP (IC₅₀ = 0.59 μM), and proving that these compounds exhibited anti-HIV activity by inhibiting the RT enzyme.

In view of the metabolic instability of the thiophene ring, the privileged structure pyrrolo[3,2-*d*]pyrimidine was introduced to replace the thiophene[3,2-*d*]pyrimidine core as an alternative scaffold, hoping that it may interact favorably with the backbone and side chain amide groups in the solvent-exposed tolerant region II and improve potency against resistance-associated variants. The results showed all compounds displayed excellent activity against WT HIV-1 strain with EC₅₀ values of 2.44–35.7 nM. In particular, **21a** exhibited prominent antiviral potency with EC₅₀ values of 2.44 nM, being more potent than the reference drug NVP (EC₅₀ = 281 nM), ETV (EC₅₀ = 4.0 nM), and AZT (EC₅₀ = 7.0 nM). Compounds **20a**, **21b**, **21c**, and **22a** were also demonstrated with equipotent potency compared to AZT with EC₅₀ values of 8.90, 5.92, 5.72, and 9.51 nM, respectively. From the results of subseries-20, subseries-21 and subseries-22, it was concluded that the order of potency of the R³ substituent was in following order: –SO₂NH₂ > –CONH₂ ≈ –SO₂CH₃, and this is consistent with our previously established SAR conclusions. Moreover, except for compound **20c**, the other eight compounds exhibited from moderate to excellent activity against mutant strain RES056 with EC₅₀ values ranging from 85 to 567 nM. Compound **21a** turned out to be the most potent inhibitor (EC₅₀ = 85 nM) against RES056, being slightly inferior to that of the reference drug ETV (EC₅₀ = 35 nM), but remarkably superior to that of NVP (EC₅₀ > 15020 nM). Replacement of H atom in the pyrrole ring with (methylsulfonyl)benzene or (methoxymethyl)benzene markedly reduced the activity toward WT HIV-1 strain as well as mutant HIV-1 strain (such as **21a** vs **20a**, **22a**; **21b** vs **20b**, **22b**; **21c** vs **20c**, **22c**), which reveals that these modifications directed to the solvent-exposed tolerant region II had limited influence on their antiviral

activity and failed to retain or enhance their activity against WT and mutant HIV-1 strains.

According to the data against mutant HIV-1 strains, **20a–c**, **21a–c**, and **22a–c** exhibited effective inhibitory activity with EC_{50} values ranging from low-micromolar concentration to single-digit nanomolar level. The activity to mutant strains exhibits a similar trend to WT strain. Compound **21a** was proved to be the most potent inhibitor, being about 1.5–2.6-fold (WT, EC_{50} = 2.44 nM; L100I, EC_{50} = 4.24 nM; Y181C, EC_{50} = 4.80 nM; F227L + V106A, EC_{50} = 17.8 nM) and 4–5-fold (K103N, EC_{50} = 1.03 nM; Y188L, EC_{50} = 7.16 nM; E138K, EC_{50} = 3.95 nM) more potent than the reference drug ETV, and remarkably superior to that of NVP and EFV. Especially, **21a** showed improved activity compared to K-5a2 (EC_{50} = 2.91 nM) and 25a (EC_{50} = 1.3 nM) against K103N. For Y181C, **21a** displayed comparable activity to K-5a2 (EC_{50} = 3.25 nM) and 25a (EC_{50} = 4.7 nM). In addition, **21a** inhibited Y188L, E138K, and F227L + V106A with up to 1.4–1-fold higher potency than 25a, but it was still inferior to that of K-5a2.

More excitingly, subseries-21 exhibited improved activity against K103N compared to against WT strain, with fold resistance (FR, ratio of EC_{50} against mutant strain/ EC_{50} against WT strain) of 0.3–0.6 (Table S2). Subseries-22 was more effective to Y181C mutant strain, and **22a–c** demonstrated more potent activity to Y181C than to WT strain (FR = 0.5–0.7). Furthermore, **22c** was also demonstrated with EC_{50} value of 33.1 nM against E138K (FR = 0.9). However, there is no obvious regularity of subseries-20. For Y181C and E138K, the activity of **20b** (EC_{50} = 19.4 and 31.6 nM) was superior and comparable to that against WT (EC_{50} = 30.8 nM), but the potency of **20a** and **20c** against all the mutant strains was inferior to that against WT strain.

Next, the inhibitory effects to WT RT were investigated. The result showed that all the compounds exhibited potent activity toward WT RT with IC_{50} values ranging from 0.075 to 0.380 μ M, being superior to NVP (IC_{50} = 0.879 μ M). Especially, the two most potent compounds **21a** and **21b** exhibited the highest enzymatic inhibition activity (IC_{50} = 0.075 and 0.098 μ M, respectively). On the whole, the SAR for inhibitory potency is in line with their results of the cellular tests, which can be explained by that the binding target of all these novel derivatives are RT and that they all acted as classical NNRTIs.

Molecular modeling analysis was carried out to rationalize the SAR results of these novel derivatives; the representative compounds **9a**, **9b**, **14c**, **21a**, and **22a** were docked into the NNIBP of HIV-1 RT (PDB: 3M8Q) by SurflexDock SYBYL-X 2.0 software. PyMOL was used to visualize the results (Figure 3 and S1). The docking protocol was described in our previous articles.^{13,14}

As shown in Figure 3 and S1, the selected compounds **9a**, **9b**, **14c**, **21a**, and **22a** were bound to the NNIBP in a similar conformation with the precursor compounds ETV and K-5a2. The interactions responsible for the high-affinity binding to the NNIBP were also observed in their molecular docking results. The most potent compound **21a** (Figure 3A) adopts typical horseshoe-like conformation at the binding site, with the left wing interacting with Tyr181 and Tyr188 and pointed toward the direction of Trp229 and Phe227. The hydrogen bond between the linker N atom and N₁ atom of the pyrrolo[3,2-d]pyrimidine with the main-chain of Lys101 is conserved. The extended sulfamide group was sandwiched between Lys104 and Val106 and developed double hydrogen bonds with their

Figure 3. Predicted binding modes of **21a** with the HIV-1 WT RT crystal structure (PDB: 3M8Q). Hydrogen bonds between inhibitors and amino acid residues are indicated with dashed lines (yellow). Ligand carbon atoms are shown in cyan and protein carbon atoms in white. Nonpolar-hydrogen atoms are not shown for clarity.

backbones, which was considered important for keeping the antiviral activities against HIV-1 mutant strains. Compared to **21a**, **9a** (Figure S1B, see in Supporting Information) lost critical hydrogen bond interactions with Lys101, and **9b** (Figure S1C) lacked double hydrogen bond interactions with Lys104 and Val106, which may account for their sharply reduced potency against mutant HIV-1 strains. As for compound **14c** (Figure S1D), although the ethynylpyridine group positioned in the chimney region formed by Phe227 and Trp229, it did not develop any interactions with the hydrophobic tunnel. Considering the cyanovinyl group of RPV could form extensive interactions with the highly conserved residue Trp229 and exhibit improved potency against resistance-associated variants than ETV, further explorations with various substitutions at the left wing may help to broaden antiviral profiles. Figure S1E demonstrated that the newly introduced (ethoxymethyl)benzene group of **22a** could extend to solvent-exposed tolerant region II, but it did not develop any novel interactions with NNIBP. In addition, it also has a poor score in the molecular docking scores function.

In conclusion, with the aim to explore solvent-exposed tolerant regions of NNIBP for seeking novel HIV-1 NNRTIs, several subseries of novel derivatives with structural modifications on the left wing, right wing, and center ring of the lead were designed based on the detailed analysis of the binding mode of K-5a2 in the NNIBP. Most target compounds inhibited HIV-1 replication in MT-4 cells with EC_{50} values in the single digit nanomolar range, especially, the preferred compound **21a** harboring single-digit nanomolar potency against WT and single mutant HIV-1 strains (EC_{50} = 1.03–7.16 nM), being superior to those of the reference drugs ETV. In addition, **21a** also exhibited improved activity against double mutant HIV-1 strain F227L + V106A (EC_{50} = 17.8 nM) compared to that of ETV (EC_{50} = 26.4 nM). Subsequent RT enzymatic assay proved that newly synthesized derivatives showed higher affinity to HIV-1 RT and acted as classical NNRTIs. Molecular simulation was conducted to understand the binding mode of interactions of the selected compounds and to give a reasonable explanation of preliminary SARs. In general, the promising activity of **21a** makes it possible for service as a drug candidate for the treatment of HIV-1 infection.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.8b00054.

Experimental protocols for synthesis and characterization of compounds, *in vitro* anti-HIV assay, and modeling study (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

* (P.Z.) E-mail: zhanpeng1982@sdu.edu.cn. Phone: 086-531-88382005.

* (X.L.) E-mail: xinyongl@sdu.edu.cn. Phone: 086-531-88380270.

ORCID

Dongwei Kang: 0000-0001-9232-953X

Zhipeng Huo: 0000-0003-3126-671X

Boshi Huang: 0000-0003-2243-1947

Peng Zhan: 0000-0002-9675-6026

Funding

Financial support from the National Natural Science Foundation of China (NSFC No. 81273354), Key Project of NSFC for International Cooperation (No. 81420108027), Young Scholars Program of Shandong University (YSPSDU No. 2016WLJH32, to P.Z.), Major Project of Science and Technology of Shandong Province (No. 2015ZDJS04001), Key research and development project of Shandong Province (No. 2017CXGC1401), and K.U. Leuven (GOA 10/014) is gratefully acknowledged.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank K. Erven, K. Uyttersprot, and C. Heens for technical assistance with the HIV assays.

■ ABBREVIATIONS

HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; DAPY, dihydropyrimidine; NNRTIs, non-nucleoside reverse transcriptase inhibitors; NNIBP, non-nucleoside inhibitor binding pocket; HAART, highly active antiretroviral therapy; RT, reverse transcriptase; SAR, structure–activity relationship; FR, fold resistance; WT, wild-type; NVP, nevirapine; DLV, delavirdine; EFV, efavirenz; ETV, etravirine; RPV, rilpivirine; AZT, azidothymidine

■ REFERENCES

- (1) Shattock, R. J.; Warren, M.; McCormack, S.; Hankins, C. A. AIDS. Turning the tide against HIV. *Science (Washington, DC, U. S.)* **2011**, *333*, 42–43.
- (2) Zhan, P.; Chen, X.; Li, D.; Fang, Z.; De Clercq, E.; Liu, X. HIV-1 NNRTIs: structural diversity, pharmacophore similarity, and implications for drug design. *Med. Res. Rev.* **2013**, *33*, E1–E72.
- (3) Li, D.; Zhan, P.; De Clercq, E.; Liu, X. Strategies for the design of HIV-1 non-nucleoside reverse transcriptase inhibitors: lessons from the development of seven representative paradigms. *J. Med. Chem.* **2012**, *55*, 3595–3613.
- (4) Zhan, P.; Pannecouque, C.; De Clercq, E.; Liu, X. Anti-HIV Drug Discovery and Development: Current Innovations and Future Trends. *J. Med. Chem.* **2016**, *59*, 2849–2878.

(5) Este, J. A.; Cihlar, T. Current status and challenges of antiretroviral research and therapy. *Antiviral Res.* **2010**, *85*, 25–33.

(6) Kang, D.; Huo, Z.; Wu, G.; Xu, J.; Zhan, P.; Liu, X. Novel fused pyrimidine and isoquinoline derivatives as potent HIV-1 NNRTIs: a patent evaluation of WO2016105532A1, WO2016105534A1 and WO2016105564A1. *Expert Opin. Ther. Pat.* **2017**, *27*, 383–391.

(7) Bec, G.; Meyer, B.; Gerard, M. A.; Steger, J.; Fauster, K.; Wolff, P.; Burnouf, D.; Micura, R.; Dumas, P.; Ennifar, E. Thermodynamics of HIV-1 reverse transcriptase in action elucidates the mechanism of action of non-nucleoside inhibitors. *J. Am. Chem. Soc.* **2013**, *135*, 9743–9752.

(8) de Bethune, M. P. Non-nucleoside reverse transcriptase inhibitors (NNRTIs), their discovery, development, and use in the treatment of HIV-1 infection: a review of the last 20 years (1989–2009). *Antiviral Res.* **2010**, *85*, 75–90.

(9) Wensing, A. M.; Calvez, V.; Gunthard, H. F.; Johnson, V. A.; Paredes, R.; Pillay, D.; Shafer, R. W.; Richman, D. D. 2015 Update of the Drug Resistance Mutations in HIV-1. *Topics in HIV medicine* **2015**, *23*, 132–141.

(10) Chen, X.; Zhan, P.; Li, D.; De Clercq, E.; Liu, X. Recent advances in DAPYs and related analogues as HIV-1 NNRTIs. *Curr. Med. Chem.* **2011**, *18*, 359–376.

(11) Chen, W.; Zhan, P.; Daelemans, D.; Yang, J.; Huang, B.; De Clercq, E.; Pannecouque, C.; Liu, X. Structural optimization of pyridine-type DAPY derivatives to exploit the tolerant regions of the NNRTI binding pocket. *Eur. J. Med. Chem.* **2016**, *121*, 352–363.

(12) Song, Y.; Fang, Z.; Zhan, P.; Liu, X. Recent advances in the discovery and development of novel HIV-1 NNRTI platforms (Part II): 2009–2013 update. *Curr. Med. Chem.* **2013**, *21*, 329–355.

(13) Kang, D.; Fang, Z.; Huang, B.; Lu, X.; Zhang, H.; Xu, H.; Huo, Z.; Zhou, Z.; Yu, Z.; Meng, Q.; Wu, G.; Ding, X.; Tian, Y.; Daelemans, D.; De Clercq, E.; Pannecouque, C.; Zhan, P.; Liu, X. Structure-Based Optimization of Thiophene[3,2-d]pyrimidine Derivatives as Potent HIV-1 Non-nucleoside Reverse Transcriptase Inhibitors with Improved Potency against Resistance-Associated Variants. *J. Med. Chem.* **2017**, *60*, 4424–4443.

(14) Kang, D.; Fang, Z.; Li, Z.; Huang, B.; Zhang, H.; Lu, X.; Xu, H.; Zhou, Z.; Ding, X.; Daelemans, D.; De Clercq, E.; Pannecouque, C.; Zhan, P.; Liu, X. Design, Synthesis, and Evaluation of Thiophene[3,2-d]pyrimidine Derivatives as HIV-1 Non-nucleoside Reverse Transcriptase Inhibitors with Significantly Improved Drug Resistance Profiles. *J. Med. Chem.* **2016**, *59*, 7991–8007.

(15) Kang, D.; Ding, X.; Wu, G.; Huo, Z.; Zhou, Z.; Zhao, T.; Feng, D.; Wang, Z.; Tian, Y.; Daelemans, D.; De Clercq, E.; Pannecouque, C.; Zhan, P.; Liu, X. Discovery of Thiophene[3,2-d]pyrimidine Derivatives as Potent HIV-1 NNRTIs Targeting the Tolerant Region I of NNIBP. *ACS Med. Chem. Lett.* **2017**, *8*, 1188–1193.

(16) Wang, L.; Tian, Y.; Chen, W.; Liu, H.; Zhan, P.; Li, D.; Liu, H.; De Clercq, E.; Pannecouque, C.; Liu, X. Fused heterocycles bearing bridgehead nitrogen as potent HIV-1 NNRTIs. Part 2: discovery of novel [1,2,4]Triazolo[1,5-a]pyrimidines using a structure-guided core-refining approach. *Eur. J. Med. Chem.* **2014**, *85*, 293–303.

(17) Huang, B.; Liang, X.; Li, C.; Chen, W.; Liu, T.; Li, X.; Sun, Y.; Fu, L.; Liu, H.; De Clercq, E.; Pannecouque, C.; Zhan, P.; Liu, X. Fused heterocycles bearing bridgehead nitrogen as potent HIV-1 NNRTIs. Part 4: design, synthesis and biological evaluation of novel imidazo[1,2-a]pyrazines. *Eur. J. Med. Chem.* **2015**, *93*, 330–337.

(18) Huang, B.; Li, C.; Chen, W.; Liu, T.; Yu, M.; Fu, L.; Sun, Y.; Liu, H.; De Clercq, E.; Pannecouque, C.; Balzarini, J.; Zhan, P.; Liu, X. Fused heterocycles bearing bridgehead nitrogen as potent HIV-1 NNRTIs. Part 3: optimization of [1,2,4]triazolo[1,5-a]pyrimidine core via structure-based and physicochemical property-driven approaches. *Eur. J. Med. Chem.* **2015**, *92*, 754–765.