Bioorganic & Medicinal Chemistry Letters 23 (2013) 6593–6597

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel piperidinylamino-diarylpyrimidine derivatives with dual structural conformations as potent HIV-1 non-nucleoside reverse transcriptase inhibitors

Xuwang Chen [a], Xin Liu [a], Qing Meng [a], Ding Wang [a], Huiqing Liu [b], Erik De Clercq [c], Christophe Pannecouque [c], Jan Balzarini [c], Xinyong Liu [a,*]

[a] Department of Medicinal Chemistry, Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Shandong University, 44, West Culture Road, 250012 Ji'nan, Shandong, PR China
[b] Institute of Pharmacology, School of Medicine, Shandong University, 44, West Culture Road, 250012 Ji'nan, Shandong, PR China
[c] Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 29 August 2013
Revised 10 October 2013
Accepted 28 October 2013
Available online 1 November 2013

Keywords:
HIV-1 NNRTIs
DAPY
Piperidin-4-yl-aminopyrimidines
Dual conformations
Molecular hybridization

ABSTRACT

A series of novel piperidinylamino-diarylpyrimidine (pDAPY) derivatives with dual structural conformations was designed through a molecular hybridization strategy and expected to bind into the non-nucleoside inhibitor binding pocket (NNIBP) of HIV-1 RT in a flexible manner. A cell-based antiviral screening assay showed that some compounds were active against both wild-type and drug-resistant mutant virus strains (K103N+Y181C RT) of HIV-1 (compound **10b3** with EC_{50} = 0.047 and 4.6 μM, selectivity index = 2145 and 22, respectively). Molecular simulation studies indicated that compound **10b3** could maintain the key hydrophobic interaction and hydrogen bonds with the NNIBP of two RT/ligand complexes. In particular, it could simultaneously occupy the protein/solvent interface and the entrance channel. Exploring these hybrid molecules with dual binding conformations might provide optional chemical scaffolds as novel HIV-1 reverse transcriptase inhibitors (HIV-1 NNRTIs).

Acquired immunodeficiency syndrome (AIDS) that is caused by human immunodeficiency virus type-1 (HIV-1), threatens human health and life and spreads rapidly worldwide because of no effective vaccine.[1] HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs), as an ingredient of highly active antiretroviral therapy (HAART), have played an indispensible role in first-line drug regimens[2] with five drugs approved by the US FDA. Especially, diarylpyrimidine (DAPY) derivatives, such as dapivirine (TMC120, **1**), etravirine (TMC125, **2**) and rilpivirine (TMC278, **3**), have been the hotspot in structural modification research over the past decade.[3] Among these structurally diverse analogues, piperidin-4-yl-aminopyrimidines (**4**) with piperidine replacing the 4-cyanophenyl group of DAPYs (Fig. 1), showed excellent activity against HIV-1 replication,[4,5] which have recently attracted our attention.[6–8]

According to the reported crystal structures of the HIV-1 RT/NNRTI complexes,[4,9] both DAPY derivatives and piperidin-4-yl-aminopyrimidines formed hydrogen bonds with Lys101, and hydrophobic interaction (π–π stacking) with a sub-pocket surrounded by Tyr181, Tyr188 and Trp229. The 4-cyanophenyl group of DAPYs and the piperidine-linked benzyl group are oriented towards the Pro236 and Val106 of the opening window of RT. Thus, a series of novel piperidinylamino-diarylpyrimidine (pDAPY) derivatives with dual structural conformations was designed through a molecular hybridization strategy[10] by introducing both a piperidine-linked benzyl group and a 4-cyanophenyl group into the central pyrimidine ring (Fig. 2). The newly designed compounds were expected to bind into the non-nucleoside inhibitor binding pocket (NNIBP) of RT according to the binding modes of the lead compounds **2** and **4**. As depicted in Figure 2, the hydrophobic interaction and the key hydrogen bonds were retained in both two modes. The piperidine-linked benzyl group and the 4-cyanophenyl group were expected to fit into the protein/solvent interface respectively close to Pro236/Val106/Leu234 and an open region in front of Lys101/Glu138/Val179 which is considered as the entrance channel[11] for the NNRTI. The present study addresses newly designed compounds with dual binding conformations, their inhibitory activity against HIV-1 replication in cell culture and the molecular simulation studies.

The newly designed compounds were synthesized by an expeditious method as depicted in Scheme 1. The intermediates **6a–b** were readily prepared from 4,6-dichloropyrimidin-2-amine (**5**) and 2,4,6-trisubstituted phenol under DMF/Cs_2CO_3 condition,[12] and intermediates **7a–b** were obtained via a palladium-catalyzed coupling reaction.[13] Treatment of **7a–b** with 4-amino-1-Boc-

* Corresponding author.
E-mail addresses: xinyongl@sdu.edu.cn, xinyongllab@163.com (X. Liu).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.10.059

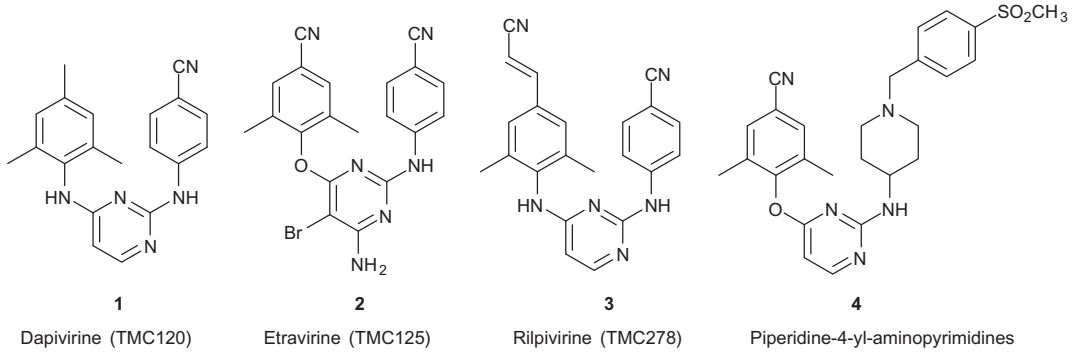


Figure 1. The structures of the HIV-1 NNRTIs.

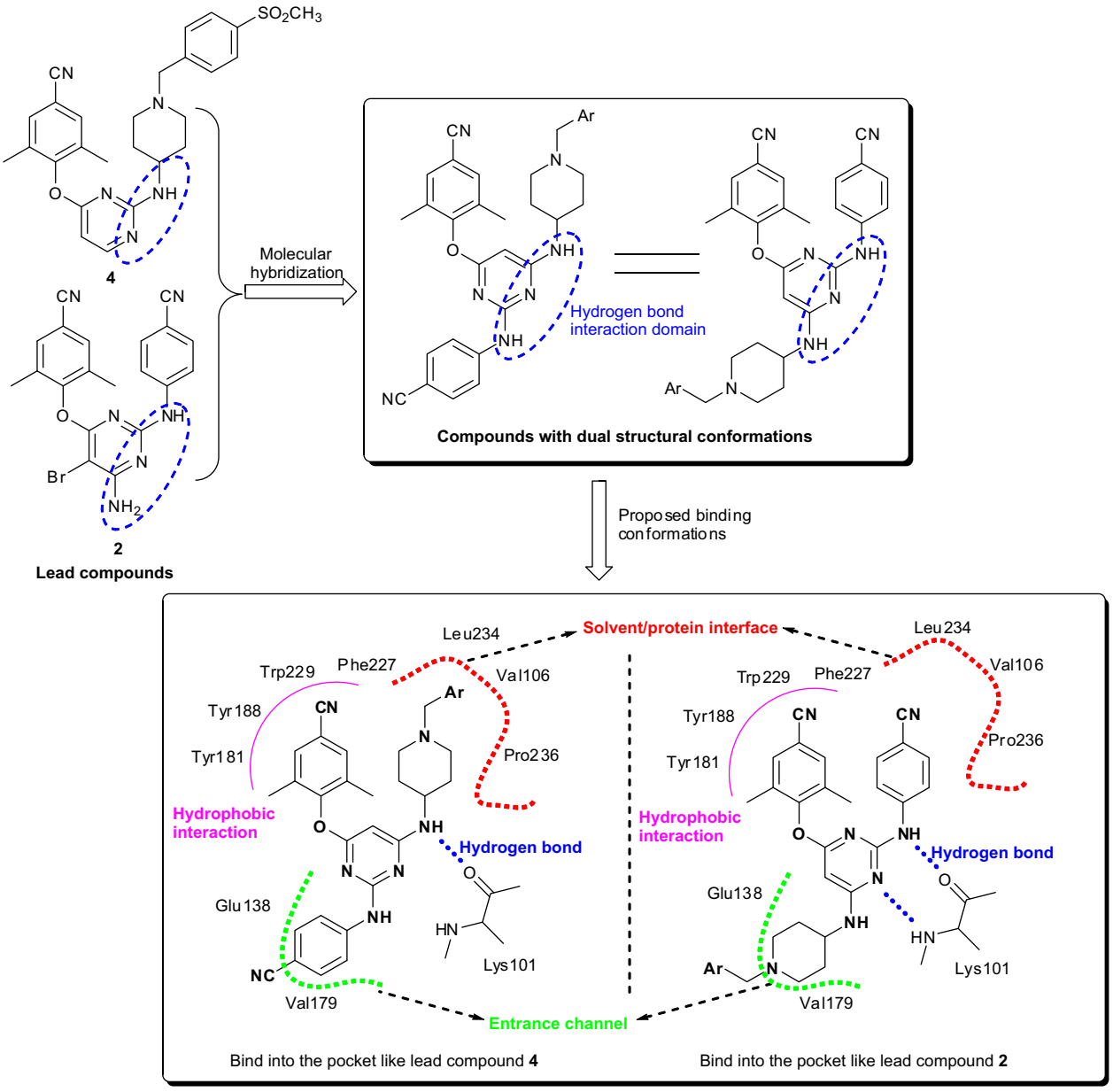
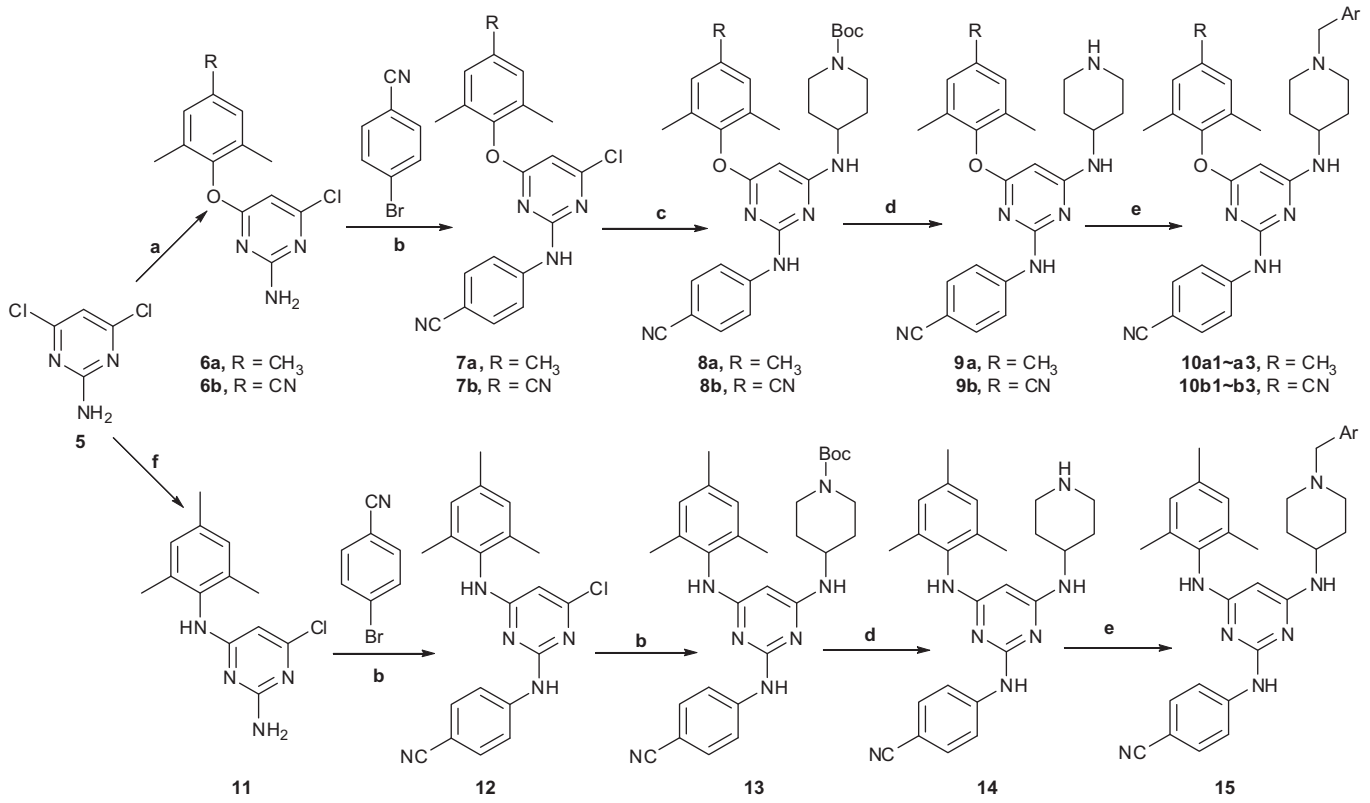


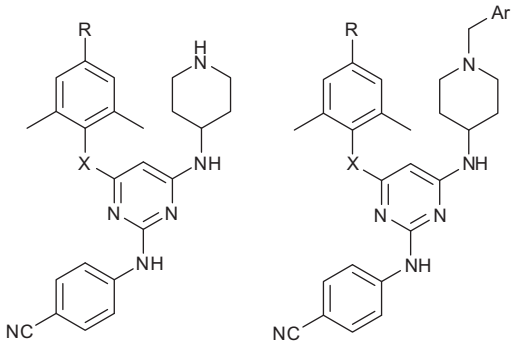
Figure 2. The design of new compounds via molecular hybridization and proposed binding conformations.

piperidine in refluxing DMSO gave the intermediate **8a**–**b**, of which the Boc group was removed in the presence of trifluoroacetic acid with satisfactory yields.[14] The exposed NH group of piperidine was linked with substituted benzyl to obtain the target compounds



Scheme 1. Reagents and conditions: (a) 2,4,6-trisubstituted phenol, Cs_2CO_3, DMF, 100 °C, overnight, 66–71%; (b) $PdAc_2$ (cat.), Xantphos (cat.), sodium *tert*-butoxide, dioxane, 80–100 °C, 6 h-overnight, 41–76%; (c) 4-amino-1-Boc-piperidine, K_2CO_3, DMSO, reflux, 11 h, 60–64%; (d) trifluoroacetic acid, CH_2Cl_2, room temperature, overnight, 84–87%; (e) substituted benzyl chloride (or 4-picolyl chloride hydrochloride), K_2CO_3, DMF, room temperature, overnight, 54–67%; (f) 2,4,6-trimethylaniline, concentrated HCl (cat.), dioxane, reflux, 20 h, 53%.

Table 1
Activity against HIV-1 (wild-type, III_B) and resistant mutant strain RES056 (K103N+Y181C RT) in MT-4 cell cultures using the MTT method



Compd	R	X	Ar	EC_{50}[a] (μM)		CC_{50}[b] (μM)	SI[c]	
				III_B	RES056		III_B	RES056
9a	CH_3	O		0.14 ± 0.05	⩾1.8	9.6 ± 10	69	⩽5
9b	CN	O		0.038 ± 0.0028	0.95 ± 0.42	4.2 ± 1.7	112	4
10a1	CH_3	O	4-SO_2NH_2-Ph	0.19 ± 0.04	>28	11 ± 5.8	61	<1
10a2	CH_3	O	4-SO_2CH_3-Ph	0.13 ± 0.08	⩾16	124 ± 49	934	⩽8
10a3	CH_3	O	4-Pyridyl	0.24 ± 0.12	>11	11 ± 9.2	44	<1
10b1	CN	O	4-SO_2NH_2-Ph	0.13 ± 0.05	3.9 ± 0.2	12 ± 6.3	93	3
10b2	CN	O	4-SO_2CH_3-Ph	0.058 ± 0.028	>206	>206	>3551	X1
10b3	CN	O	4-Pyridyl	0.047 ± 0.011	4.6 ± 2.3	100 ± 27	2145	22
15	CH_3	NH	4-SO_2NH_2-Ph	0.077 ± 0.065	>6.4	6.4 ± 6.1	83	<1
NVP				0.17 ± 0.057	2.5 ± 1.0	>15	>89	>6
DLV				0.13 ± 0.043	>36	>36	>227	X1
EFV				0.007 ± 0.003	0.52 ± 0.02	>6.3	>855	>12

[a] EC_{50}: concentration of compound required to achieve 50% protection of MT-4 cell cultures against HIV-1-induced cytopathicity.
[b] CC_{50}: cytotoxic concentration of compound that reduces the normal uninfected cell viability by 50%.
[c] SI: selectivity index, the ratio of CC_{50}/EC_{50}. X1 stands for ⩾1 or <1.

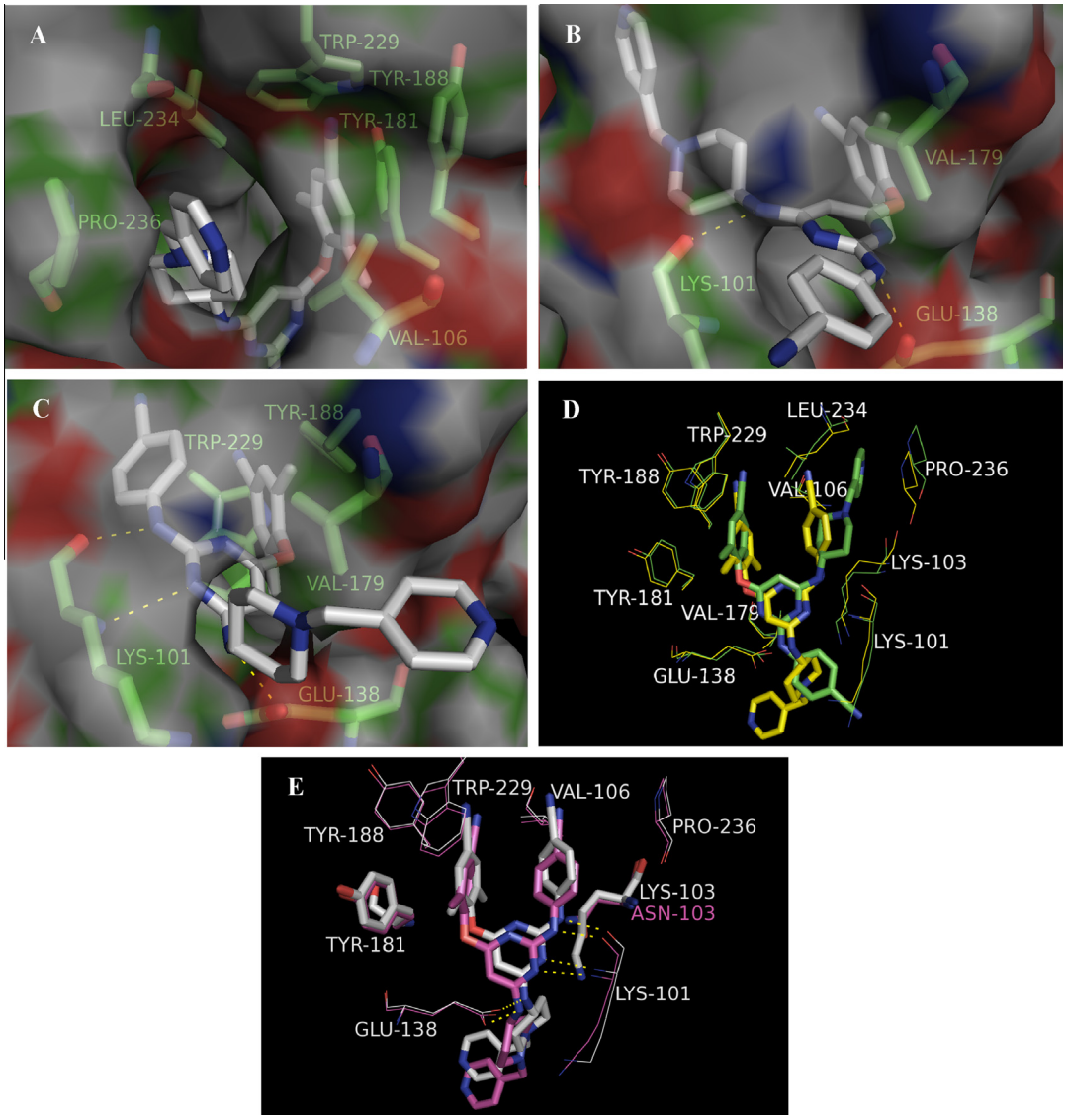


Figure 3. Predicted binding modes of compound **10b3** in the NNIBP of HIV-1 RT showed by PyMOL:[17] (A) and (B) $RT_{wild\text{-}type}$ (PDB code: 3M8Q);[4] (C) $RT_{wild\text{-}type}$ (PDB code: 3MEC);[9] (D) Superimposed conformation of the two binding modes (green, PDB code: 3M8Q; yellow, PDB code: 3MEC); (E) Superimposed conformations of $RT_{wild\text{-}type}$ (white, PDB code: 3MEC)[9] and RT_{K103N} (magenta, PDB code: 3MED).[9] Hydrogen bonds are indicated with dashed lines in yellow and hydrogen atoms are omitted for the sake of clarity.

10a1–a3 and **10b1–b3**. Compound **15** was prepared via a substitution reaction of starting material **5** and 2,4,6-trimethylaniline catalyzed by concentrated hydrochloric acid, and then followed a similar procedure as in the preparation of compounds **10a1–a3**.

The title compounds including intermediates **9a** and **9b** were tested in a cell-based antiviral assay against HIV-1 (wild-type, III_B and a resistant mutant HIV-1 strain, containing K103N+Y181C in the RT) with non-nucleoside drugs nevirapine (NVP), delavirdine mesylate (DLV) and efavirenz (EFV) as reference drugs.[6–8,15] All the new compounds were active against wild-type HIV-1 with EC_{50} values in the low sub-micromolar concentration range (Table 1). Compounds **10a1–a3** and **9a** with a 2,4,6-trimethylphenyl group (R = CH_3) showed no activity against the frequently encountered drug-resistant mutant strain (containing the K103N+Y181C RT).[16] After *para*-methyl was replaced by a cyano group (R = CN), compounds (**9b** and **10b1–b3**) not only showed clearly improved activity against wild-type HIV-1 but also had the ability to inhibit the mutant virus strain except for compound **10b2**. Introduction of polar hydrophilic substituents (4-SO_2NH_2-Ph, 4-SO_2CH_3-Ph and pyridin-4-yl) at the N atom of the piperidine ring decreased the activity by comparing compound **9b** with **10b1–b3**, but lowered the cytotoxicity (CC_{50} values) in MT-4 cell cultures. Compared to **10a1**, compound **15** with a –NH– linker (X = NH) was more active against wild-type HIV-1, but still not effective towards the drug-resistant mutant virus strain. Overall, **10b3** was confirmed as a promising compound with considerable antiviral potency (EC_{50} = 0.047 (wild-type) and 4.6 μM (mutant)), low cytotoxicity (CC_{50} = 100 μM) and high selectivity (SI = 2145 and 22), respectively, for the wild-type and mutant HIV-1 strains.

An HIV-1 RT inhibitory assay was performed via an ELISA assay using a commercial kit (using poly rA:dT as the template/primer). The result indicated that the representative compound **10b3** showed moderate affinity to HIV-1 RT, and inhibited the activity of RT in vitro (IC_{50} = 33 μM), which was less potent than the reference drugs NVP (IC_{50} = 0.51 μM) and etravirine (IC_{50} = 0.12 μM).

To further check the mode of interaction, compound **10b3** was docked into two RT/ligand complexes PDB 3M8Q (the initial ligand is lead compound **4**)[4] and PDB 3MEC (the initial ligand is lead

compound **2**)[9] using the software Surflex–Dock SYBYL-X. Apparently, compound **10b3** displayed some well-known interactions with NNIBP in both two docking studies (Fig. 3A and D): the π–π interaction to the side chain phenyl of Tyr181 and the edge-to-face interaction to the tail ring of the highly conserved Trp229. The following detailed analysis of the docking results revealed that the hybrid molecule **10b3** had achieved the main interactions as designed.

(1) Similar to lead compound **4**,[4] the 1-(pyridin-4-ylmethyl)piperidine group occupied the groove lined by Pro236, Val106 and Leu234 and extended the polar 4-pyridyl head near the solvent/protein interface (Fig. 3A) and the NH moiety at the 4-position of piperidine formed hydrogen-bonding interaction with the Lys101 backbone carbonyl (Fig. 3B). The 4-cyanophenyl group was oriented into the entrance channel[11] in front of Lys101, Glu138 and Val179, and the NH group between the pyrimidine ring and the 4-cyanophenyl also formed a H-bond with Glu138 (Fig. 3B).
(2) Similar to lead compound **2**,[9] the 2-position NH linker and the N atom of the pyrimidine ring interacted with the Lys101 backbone carbonyl and the α-amino, forming double H-bonds. The newly introduced 1-(pyridin-4-ylmethyl) piperidine group stretched out in the NNIBP through the entrance channel (near Lys101, Glu138 and Val179) and formed a hydrogen-bonding interaction between Glu138 and the NH moiety at 4-position of the piperidine (Fig. 3C).
(3) The superimposed conformation of the above two binding modes was showed in Figure 3D (green: the mode similar to lead compound **4**; yellow: the mode similar to lead compound 2). The Tyr181, Tyr188 and Trp229 were kept nearly the same conformation, whereas, the Lys103, Leu234 and Pro236 showed different poses between the two modes. The 1-(pyridin-4-ylmethyl)piperidine group and 4-cyanophenyl group fitted into the protein/solvent interface and an open region of the entrance channel respectively in both two modes.

In addition, compound **10b3** was also docked into the NNIBP of resistant mutant RT_{K103N} (PDB code: 3MED).[9] Through the superimposed conformations of mutant and wide-type RT in Figure 3E, it was observed that the ligand had similar binding interactions with NNIBP except for some minor differences. The Tyr181 of both mutant (magenta) and the wild-type (white) RT kept nearly the same orientation and was parallel to the 4-cyano-2,6-dimethylphenyl group of compound **10b3**. When the resistant mutation at the Tyr181 side-chain emerged (usually into Cys181), the π–π interaction got lost. Moreover, the ligand formed double H-bonds with Lys101 even when the Lys103 (white) got mutated to Asn103 (magenta), meaning that the ligand did not interact with Lys103 or Asn103. Based on the above two aspects, in order to improve the activity against the frequently encountered drug-resistant mutant virus strain (K103N+Y181C RT), further designed compounds should strengthen π–π interactions with Tyr188 and Trp229 to weaken the dependence on Tyr181. Also, some functional groups should be introduced as potential hydrogen bond acceptor or donor to form an additional H-bond with Lys103.

In summary, based on a molecular hybridization strategy, we have designed and synthesized a novel series of HIV-1 NNRTIs that assume dual binding conformations by integration of the pharmacophoric groups respectively from piperidin-4-yl-aminopyrimidines and DAPY derivatives. The representative hybrid molecule **10b3** showed good antiviral potency against wild-type and mutant strains of HIV-1 in cell culture (EC_{50} = 0.047 and 4.6 μM). Molecular simulation studies supported our initial design hypothesis that the hybrid compounds could occupy the protein/solvent interface and the entrance channel simultaneously. Further studies on optimization of this series of compounds are currently underway, such as decreasing the molecular size and introducing other potential functional groups. It is expected that the exploration of the unique molecules with dual binding conformations will provide optional chemical scaffolds in the future design of HIV-1 NNRTIs.

Acknowledgments

The financial support from the National Natural Science Foundation of China (NSFC Nos. 81273354, 81102320, 30873133, 30772629, 30371686), Key Project of NSFC for International Cooperation (No. 30910103908), Research Fund for the Doctoral Program of Higher Education of China (Nos. 20110131130005, 20110131120037), Natural Science Foundation of Shandong province (ZR2009CM016), Independent Innovation Foundation of Shandong University (IIFSDU, No. 2010GN044), Shandong Postdoctoral Innovation Science Research Special Program (No. 201002023), China Postdoctoral Science Foundation funded project (No. 20100481282, 2012T50584) and KU Leuven (GOA 10/014) is gratefully acknowledged.

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

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.10.059.

References and notes

1. UNAIDS Report on the Global AIDS Epidemic **2012**.
2. De Clercq, E. *Curr. Med. Chem.* **2001**, *8*, 1543.
3. Chen, X. W.; Zhan, P.; Li, D. Y.; De Clercq, E.; Liu, X. Y. *Curr. Med. Chem.* **2011**, *18*, 359.
4. Kertesz, D. J.; Brotherton-Pleiss, C.; Yang, M. M.; Wang, Z. G.; Lin, X. F.; Qiu, Z. X.; Hirschfeld, D. R.; Gleason, S.; Mirzadegan, T.; Dunten, P. W.; Harris, S. F.; Villasenor, A. G.; Hang, J. Q.; Heilek, G. M.; Klumpp, K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4215.
5. Tang, G. Z.; Kertesz, D. J.; Yang, M. M.; Lin, X. F.; Wang, Z. G.; Li, W. T.; Qiu, Z. X.; Chen, J. L.; Mei, J. H.; Chen, L.; Mirzadegan, T.; Harris, S. F.; Villasenor, A. G.; Fretland, J.; Fitch, W. L.; Hang, J. Q.; Heilek, G.; Klumpp, K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6020.
6. Chen, X.; Zhan, P.; Liu, X.; Cheng, Z.; Meng, C.; Shao, S.; Pannecouque, C.; De Clercq, E. *Bioorg. Med. Chem.* **2012**, *20*, 3856.
7. Chen, X.; Zhan, P.; Pannecouque, C.; Balzarini, J.; De Clercq, E.; Liu, X. *Eur. J. Med. Chem.* **2012**, *51*, 60.
8. Chen, X.; Li, Y.; Ding, S.; Balzarini, J.; Pannecouque, C.; De Clercq, E.; Liu, H.; Liu, X. *ChemMedChem* **2013**, *8*, 1117.
9. Lansdon, E. B.; Brendza, K. M.; Hung, M.; Wang, R.; Mukund, S.; Jin, D.; Birkus, G.; Kutty, N.; Liu, X. *J. Med. Chem.* **2010**, *53*, 4295.
10. Viegas-Junior, C.; Danuello, A.; da Silva Bolzani, V.; Barreiro, E. J.; Fraga, C. A. *Curr. Med. Chem.* **2007**, *14*, 1829.
11. Ekkati, A. R.; Bollini, M.; Domaoal, R. A.; Spasov, K. A.; Anderson, K. S.; Jorgensen, W. L. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1565.
12. Ye, Y. L.; Zhou, Z.; Zou, H. J.; Shen, Y.; Xu, T. F.; Tang, J.; Yin, H. Z.; Chen, M. L.; Leng, Y.; Shen, J. H. *Bioorg. Med. Chem.* **2009**, *17*, 5722.
13. Jones, C. D.; Andrews, D. M.; Barker, A. J.; Blades, K.; Byth, K. F.; Finlay, M. R.; Geh, C.; Green, C. P.; Johannsen, M.; Walker, M.; Weir, H. M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6486.
14. Palani, A.; Shapiro, S.; McBriar, M. D.; Clader, J. W.; Greenlee, W. J.; Spar, B.; Kowalski, T. J.; Farley, C.; Cook, J.; van Heek, M.; Weig, B.; O'Neill, K.; Graziano, M.; Hawes, B. *J. Med. Chem.* **2005**, *48*, 4746.
15. Pannecouque, C.; Daelemans, D.; De Clercq, E. *Nat. Protoc.* **2008**, *3*, 427.
16. D'Aquila, R. T.; Schapiro, J. M.; Brun-Vezinet, F.; Clotet, B.; Conway, B.; Demeter, L. M.; Grant, R. M.; Johnson, V. A.; Kuritzkes, D. R.; Loveday, C.; Shafer, R. W.; Richman, D. D. *Top. HIV Med.* **2003**, *11*, 92.
17. *The PyMOL Molecular Graphics System version 0.99*; DeLano Scientific: San Carlos, CA, U.S.A., 2002.