Synthesis and anti-HIV activity of 2-naphthyl substituted DAPY analogues as non-nucleoside reverse transcriptase inhibitors

ARTICLE *in* BIOORGANIC & MEDICINAL CHEMISTRY · JULY 2010

Impact Factor: 2.79 · DOI: 10.1016/j.bmc.2010.05.036 · Source: PubMed

9 AUTHORS, INCLUDING:

Qiu-Qin He
Fudan University
23 PUBLICATIONS **126** CITATIONS
SEE PROFILE

Christophe Pannecouque
University of Leuven
435 PUBLICATIONS **7,261** CITATIONS
SEE PROFILE

Bioorganic & Medicinal Chemistry 18 (2010) 4601–4605

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis and anti-HIV activity of 2-naphthyl substituted DAPY analogues as non-nucleoside reverse transcriptase inhibitors

Yong-Hong Liang [a], Qiu-Qin He [a], Zhao-Sen Zeng [a], Zhi-Qian Liu [a], Xiao-Qing Feng [a], Fen-Er Chen [a,b,*], Jan Balzarini [c], Christophe Pannecouque [c], Erik De Clercq [c]

[a] Department of Chemistry, Fudan University, Shanghai 200433, People's Republic of China
[b] Institute of Biomedical Science, Fudan University, Shanghai 200433, People's Republic of China
[c] Rega Institute for Medical Research, Katholieke Universiteit Leuven, 10 Minderbroedersstraat, B-3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 20 April 2010
Revised 13 May 2010
Accepted 14 May 2010
Available online 20 May 2010

Keywords:
HIV-1
Reverse transcriptase
NNRTIs
DAPY

ABSTRACT

Nine newly 6-cynao-2-naphthyl substituted diarylpyrimidines (DAPY) were synthesized as non-nucleoside reverse transcriptase inhibitors on the basis of our previous work. The antiviral and cytotoxicity evaluation indicated that these compounds displayed strong activity against wild-type HIV-1 at nanomolar concentrations with selectivity index SI greater than 23 779. The most active compounds **3c** and **3e** exhibited activity against the double mutant (103N+181C) strains at an EC_{50} of 0.16 and 0.15 μM, and were more activity than that of efavirenz.

1. Introduction

The development of new and more potent mutation-resistant non-nucleoside reverse transcriptase inhibitors (NNRTIs) is still an arduous task for the treatment of the HIV-1-infected patients due to the drug compliance, adverse effects and cross-resistance. Recently, we designed and synthesized a novel class of the naphthyl-substituted DAPY analogues[1–3] with strong activity against the HIV-1 LAI virus and high selectivity index, as compared to two lead compounds TMC278 and TMC125 (Fig. 1)[4–7] The structure–activity relationship (SAR) and theoretical calculated studies[1,3] have revealed that the cyano group at position *C*-6 on the naphthalene ring plays a vital role in the activity against the wild-type and double mutant HIV-1 strains, and the dual-substituted compounds at positions *C*-1 and *C*-3 on the naphthalene ring exhibit more potent activity against the mutant virus than the non- or mono-substituted compounds.

In continuation of our efforts in improving the anti-HIV activity of the naphthyl-substituted DAPY analogues against the clinically relevant HIV-1 mutant strains, nine newly 6-cyano-2-naphthyl substituted DAPY analogues were designed and synthesized.

2. Results and discussion

2.1. Chemistry

The target compounds were synthesized via the short route detailed in Scheme 1 according to our previously reported protocol.[2] The 4-chloropyrimidines **1** were treated with the 6-cyano-2-naphthol derivatives **2a–e** at 110 °C in the presence of DMF to provide the 6-cyano-2-naphthyl substituted DAPY compounds **3a–i**. The spectroscopic data of the target compounds **3a–i** were consistent with the structures shown in Scheme 2.

The intermediate 6-cyano-2-naphthol derivatives **2a–e** were synthesized using the method as shown in Scheme 2. Following the reported method, the intermediate 6-cyano-2-naphthol (**5**)

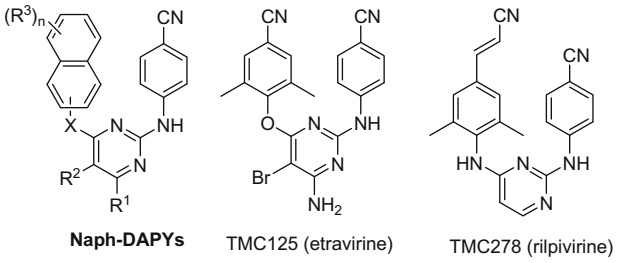
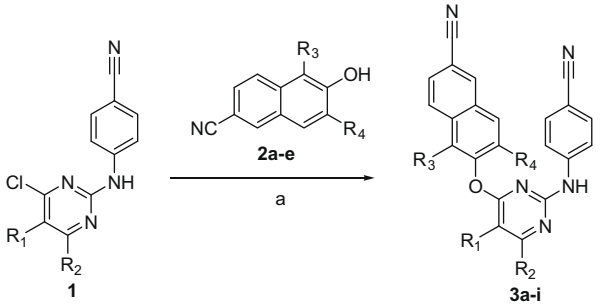


Figure 1. The chemical structures of NNRTIs.

* Corresponding author. Tel.: +86 21 65643809; fax: +86 21 65643811.
E-mail address: rfchen@fudan.edu.cn (F.-E. Chen).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.05.036



Scheme 1. Reagents and conditions: (a) K_2CO_3, DMF, 110 °C, 8–12 h, N_2.

was synthesized from 6-cyano-2-naphthol (**4**). Then, the treatment of the intermediate **5** with Br_2 yielded 1-bromo-6-cyano-2-naphthol (**6a**). The 1,3-dibromo-6-cyano-2-naphthol (**6b**) was synthesized using the reported method for 1,3,6-tribromo-2-naphthol.[8,9] Reaction of **6b** with Sn provided 3-bromo-6-cyano-2-naphthol (**6c**). Then, the methoxy-substituted 6-cyano-2-naphthol derivatives **2a–c** were prepared by the methoxylation of the corresponding bromo-substituted 6-cyano-2-naphthol compounds **2a–c**. The treatment of **5** and **2c** with NCS in the presence of NaH provided the 1-chloro-substituted naphthol compounds **2d–e**.[10]

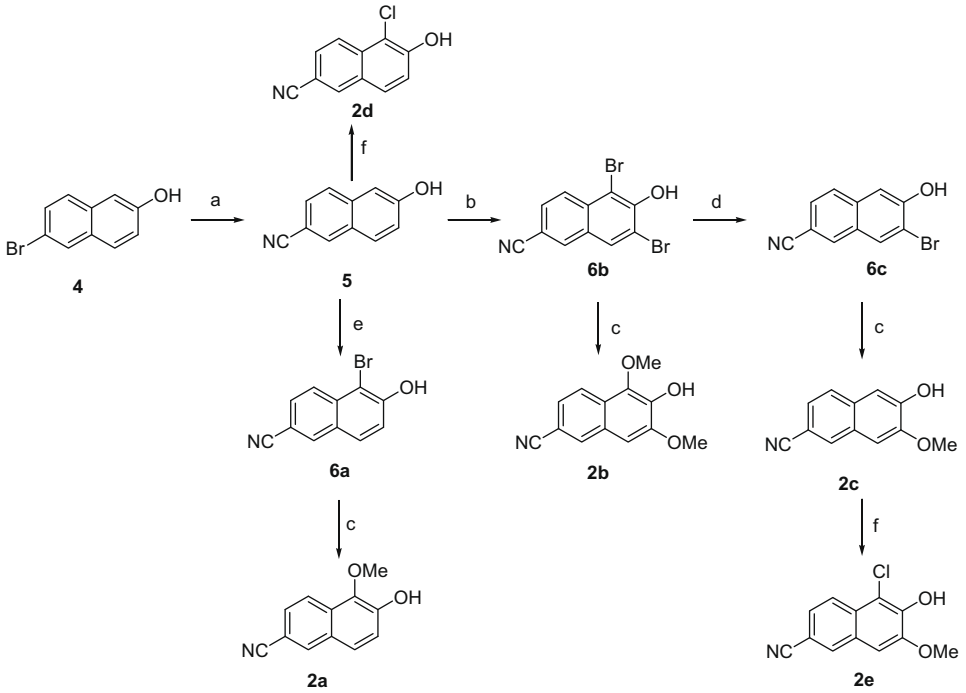
2.2. Biological activity

According to the MTT method,[11,12] the newly synthesized compounds **3a–i** were evaluated for antiviral and cytotoxicity activity in MT-4 cells infected with the HIV-1 (LAI strain, IIIB) wild-type virus and HIV-1 double mutant virus K103N+Y181C (lysine replaced at position 103 by asparagine, and tyrosine at position 181 by cysteine), in comparison with three FDA-approved drugs nevirapine, delavirdine, and efavirenz used as reference compounds.

To lend support to the status of naphthyl-substituted DAPY analogues as NNRTIs, an in vitro steady-state RT inhibition assay for three representative compounds **3a–c** was performed (Table 1). The results strongly support all of the derivatives in this study as NNRTIs, as they have no possibility of being phosphorylated for NRTI activity against RT. As shown in Table 1, the 6-cyano-2-naphthyl substituted DAPY derivatives displayed strong inhibitory activity against wild-type HIV-1 at nanomolar concentration level (EC_{50} = 0.012–0.002 μM) and low cytotoxicity (CC_{50} = 118.44–331.56 μM), resulting in high selectivity index (SI) values of 23,779–158,228.

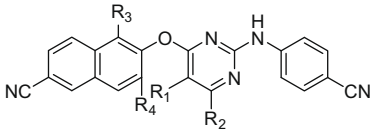
The mono-substituted derivative **3a**, **3b** and **3d** exhibited an excellent potency against wild-type virus (EC_{50} = 0.002 and 0.006 μM), and the compounds **3a** and **3d** displayed a moderate activity against the double mutants virus K103N+Y181C and were more potent compared to the non-substituted compound **3j**.[3] However, the 3-methyoxy-substituted compound **3b** displayed no activity against the double mutant form, nor did the previously reported 3-bromo-substituted compound **3l**.[3] Introduction of double substituted R_3 and R_4 groups at positions *C*-1 and *C*-3 of the naphthyl ring was also investigated; the compounds **3c** and **3e** showed activity against wild-type HIV-1 at an EC_{50} value of 0.005 and 0.007 μM, respectively. The potency against the double mutant virus K103N+Y181C was slightly increased compared to compound **3a**. The compounds **3f** and **3h** with a methyl-substituted pyrimidine ring were much less active against the double mutant virus than the corresponding non-substituted compound **3j** and **3k**.

In order to investigate the binding model of the designed dual-substituted compound with RT, a molecular docking study was performed using the program AUTODOCK 4.0.1[13] according to the previous reported procedures.[3] Figure 2 shows the theoretical binding model of compounds 1u and **1cam** to the non-nucleoside inhibitor binding pocket (NNIBP) of HIV-1 RT. As the previous theoretical binding model of compound **3j** with RT,[3] the dual–substituted compound **3c** adopt a horseshoe conformation in the NNIBP and the left 'wing' naphthalene ring exhibits strong π–π interactions with five residues (Tyr181, Tyr188, Phe227, and Trp229) surrounding the NNIBP.



Scheme 2. Reagents and conditions: (a) CuCN, DMF, reflux, 2 h; (b) (i) Br_2, AcOH, AcONa, rt, 2 h; (ii) $SnCl_2(H_2O)_2$, AcOH, 5 h; (c) MeONa, CuI, DMF, 110 °C, 3 h; (d) Sn, HCl, AcOH, reflux, 4 h; (e) Br_2, AcOH, rt, 1 h; (f) NCS, NaH, THF, reflux, 4 h.

Table 1
Anti-HIV-1 activity and cytotoxicity of compounds **3a–i** in MT-4 cells



Compd	R_1	R_2	R_3	R_4	IC_{50}[a] (μg/mL)	EC_{50}[b] (μM)		CC_{50}(μM)[c]	SI[d]
						WT(IIIB)	103 N+181C		
3a	H	H	OMe	H	0.9	0.002	0.38	>211.45	⩾134,032
3b	H	H	H	OMe	0.52	0.002	318. 07	>318.07	>158,228
3c	H	H	OMe	OMe	0.04	0.005	0.16	>118.44	⩾25,000
3d	H	H	Cl	H	nd	0.006	0.70	268.79	47,964
3e	H	H	Cl	OMe	nd	0.007	0.15	>190.65	⩾25,701
3f	Me	H	H	H	nd	0.007	331.56	>331.56	>44,856
3g	Me	H	Br	H	nd	0.012	0.83	>274.12	>23,779
3h	H	Me	H	H	nd	0.003	331.56	>331.56	>96,451
3i	H	Me	Br	H	nd	0.007	0.22	>274.12	>40,068
3j[3]	H	H	H	H	3.17	0.003	6.30	>68.80	20,548
3k[3]	H	H	Br	H	1.33	0.002	0.24	282.63	181,247
3l[3]	H	H	H	Br	0.50	0.001	>56.53	>56.53	50,357
NEV					0.37	0.075	>15.02	>15.02	>200
DEV					nd	0.072	>3.62	>3.62	50
EFV					nd	0.003	0.56	>63.36	>2112

[a] Concentration required to inhibit by 50% the in vitro RNA-dependent DNA polymerase activity of recombinant RT.
[b] Effective concentration required to protect the cell against viral cytopathicity by 50% in MT-4 cells.
[c] Concentration of compound that reduces normal uninfected MT-4 cell viability by 50%.
[d] Selectivity index: ratio CC_{50}/EC_{50} (wild-type).

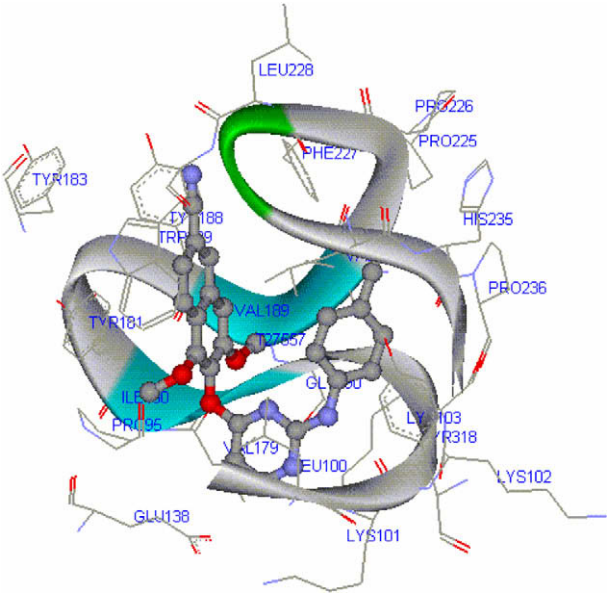


Figure 2. Binding model of compounds **3c** in the NNIBP. This figure was prepared using the programs AUTODOCKTOOLS-1.5.0 and VIEWERLITE.

3. Conclusion

In summary, we synthesized a novel series of 2-naphthyl substituted DAPYs. The biological test results indicated that the target compounds showed potent antiviral activity with EC_{50} values in the low-nanomolar concentration range and high SI value of 23,779–158,228. The double substituted compounds **3c** and **3e** displayed an excellent potency against the double mutant (103N+181C) strains at an EC_{50} of 0.16 and 0.15 μM and were more active compared to efavirenz. The results form a solid basis for continued exploration of the DAPY family of RT inhibitors.

4. Experimental section

4.1. Chemistry

Melting points were measured on a WRS-1 digital melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra on a Bruker AV 400 MHz spectrometer were recorded in DMSO-d_6. Chemical shifts are reported in δ (ppm) units relative to the internal standard tetramethylsilane (TMS). Mass spectra were obtained on a Agilent MS/5975 mass spectrometer. Elemental analyses were performed on a CARLOERBA 1106 instrument and the results of elemental analyses for C, H, and N were within (0.4% of the theoretical values. All chemicals and solvents used were of reagent grade and were purified and dried by standard methods before use. All air-sensitive reactions were run under a nitrogen atmosphere. All the reactions were monitored by TLC on pre-coated silica gel G plates at 254 nm under a UV lamp using ethyl acetate/hexane as eluent. Flash chromatography separations were obtained on silica gel (300–400 mesh).

4.1.1. Preparation of 6-hydroxy-2-naphthonitrile (5)

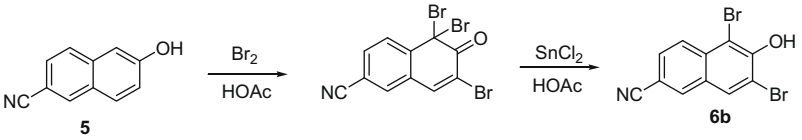
A mixture of 6-bromo-2-naphthol (32 g, 143 mmol), CuCN (20 g, 0.225) and DMF (80 mL) was stirred at 160 °C for 4 h. To the resulting mixture was added 10% NaOH (200 mL), and then insoluble material was filtered and washed with water (100 mL). The filtrate and washings were combined and filtered again. The filtrate was acidified with 10% HCl to pH 2–3 and stirred at room temperature for 30 h. Precipitated brown solids were collected by filtration to give 21 g of **5**. This crude brown solid was dried and used in the next reaction without further purification.

4.1.2. Preparation of 5-bromo-6-hydroxy-2-naphthonitrile (6a)

6-hydroxy-2-naphthonitrile **5** (6.8 g, mmol) were dissolved in glacial acetic acid (112 mL) with heating. The solution was then cooled to 25 °C and bromine (33.4 g, 209.0 mmol) was added over 30 mins. The reaction was stirred for 2 h at room temperature. Yield 94%; mp 181.4–182.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ

7.63–7.66 (m, 1H), 7.75 (d, 1H J = 9.2 Hz), 7.85 (d, 1H J = 9.2 Hz), 8.08 (d, 1H J = 9.2 Hz), 8.23 (s, 1H), 10.88 (s, 1H, OH).

4.1.3. Preparation of 5,7-dibromo-6-hydroxy-2-naphthonitrile (6b)



Sodium acetate (15 g, 182.9 mmol) and 6-hydroxy-2-naphthonitrile **5** (6.8 g, 40.2 mmol) were dissolved in glacial acetic acid (112 mL) with heating. The solution was then cooled to 0 °C and bromine (33.4 g, 209.0 mmol) was added in five portions over 10 min. The reaction was stirred for 2 h at room temperature. Ice was added to the reaction causing the formation of a light yellow precipitate, which was collected on a coarse glass frit and washed with cold water, followed by petroleum ether and left to air dry. A suspension of the yellow power 5,5,7-tribromo-6-oxo-5,6-dihydronaphthalene-2-carbonitrile and $SnCl_2(H_2O)_2$ in glacial acetic acid (150 mL) was stirred overnight at room temperature, then 15% HCl (50) was added to the reaction mixture causing the formation of a white precipitate, which was filtered and washed with cold water. The white crude solid was recrystallized from EtOH, yield **6b** as a yellow solid. Mp 210.8–211.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (m, 1H), 8.12 (d, 1H, J = 8.8 Hz), 8.46 (s, 1H), 8.51 (s, 1H), 10.92 (s, 1H, OH).

4.1.4. Preparation of 7-bromo-6-hydroxy-2-naphthonitrile (6c)

A suspension of 5, 7-dibromo-6-hydroxy-2-naphthonitrile (8 g, **6b**) and Sn (15 g) in the mixture of 150 mL EtOH and 20 mL concd HCl was refluxed for 4 h, then poured the reaction mixture into cold water (250 mL) and obtained the white solid. Yield 71%; mp 237.4–238.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.36 (s, 1H), 7.63–7.66 (m, 1H), 7.87 (d, 1H, J = 8.4 Hz), 8.31 (s, 1H), 8.36 (s, 1H), 11.23 (s, 1H, OH).

4.1.5. General procedure for the preparation of 2a–c

A suspension of **6a–c** (4 mmol), sodium methoxide (20 mmol) and CuI (1 mmol) in DMF (50 mL) was heated to 120 °C for 3 h, then poured the reaction mixture into cold water (150 mL). After filtering, the filtrate was added to 5% HCl (100 mL) and was stirred for 30 min.

4.1.5.1. 6-Hydroxy-5-methoxy-2-naphthonitrile (2a). Yield 74%; mp 143.2–144.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.85 (s, 3H, OCH_3), 7.33 (d, 1H, J = 8.8 Hz), 7.64–7.67 (m, 1H), 8.02 (d, 1H, J = 8.8 Hz), 8.41 (d, 1H, J = 1.2 Hz), 11.00 (s, 1H, OH).

4.1.5.2. 6-Hydroxy-5,7-dimethoxy-2-naphthonitrile (2b). Yield 64%; mp 125.5–125.9 °C; δ ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.85 (s, 3H, CH_3), 3.93 (s, 3H, CH_3), 7.28 (s, 1H), 7.56 (d, J = 12 Hz, 1H), 7.95 (d, J = 12 Hz, 1H), 8.29 (s, 1H), 9.79 (s, 1H, OH).

4.1.5.3. 6-Hydroxy-7-methoxy-2-naphthonitrile (2c). Yield 72%; mp 164.2–165.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.91 (s, 3H, CH_3), 7.24 (s, 1H), 7.41 (s, 1H), 7.49 (d, 1H, J = 1.6 Hz), 7.77 (d, 1H, J = 1.2 Hz), 8.24 (s, 1H), 10.24 (s, 1H, OH).

4.1.6. General procedure for the preparation of 2d–e

Sodium hydride (10 mmol) was added to a solution of **2a** or **2c** (3 mmol) in anhydrous THF (50 mL) and stirred for 5 min. Then NCS (3 mmol) was added to the reaction mixture and was refluxed for 4 h. Then the reaction mixture poured the reaction into 200 mL cold water, and the resulting brown precipitate was filtered off. The crude brown solid **2d–e** was dried to be used in the next step without further purification.

4.1.6.1. 5-Chloro-6-hydroxy-2-naphthonitrile (2d). Yield 67%; mp 186.4–187.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.43 (d, 1H, J = 8.8 Hz), 7.84 (d, 1H, J = 8.8 Hz), 7.92 (d, 1H, J = 8.8 Hz), 8.12 (d, 1H, J = 8.8 Hz), 8.51 (s, 1H), 11.13 (s, 1H, OH).

4.1.6.2. 5-Chloro-6-hydroxy-7-methoxy-2-naphthonitrile (2e). Yield 73%; mp 183.4–184.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.98 (s, 3H, CH_3), 7.49 (s, 1H), 7.69 (d, 1H, J = 8.8 Hz), 8.03 (d, 1H, J = 8.8 Hz), 8.36 (s, 1H), 10.59 (s, 1H, OH).

4.1.7. General procedure for the preparation of 3a–i

Potassium carbonate (10 mmol) was added to a solution of β-naphthol derivatives (2 mmol) in 20 mL of anhydrous DMF and was stirred for 5 min. Then 4-(4-chloro-pyrimidin-2-ylamino)benzonitrile **6** (2 mmol) was added. The reaction mixture was heated to 80 °C under nitrogen atmosphere for 8–12 h. Next, the mixture was treated with cold water (200 mL), and the resulting precipitate was filtered off. The crude products **3a–i** were recrystallized from 1,4-dioxane or EtOAc.

4.1.7.1. 6-(2-(4-Cyanophenylamino)pyrimidin-4-yloxy)-5-methoxy-2-naphthonitrile (3a). Yield 89.3%; recrystallized from AcOEt, mp 249.2–250.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.90 (s, 3H, CH_3), 6.76 (d, 1H, J = 4.0 Hz, CH), 7.25–7.55 (m, 4H, Ph), 7.64–8.50 (m, 5H, naph), 8.71 (s, 1H, CH), 10.10 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 61.77, 99.70, 102.55, 108.66, 118.22, 118.99, 119.27, 123.33, 125.13, 126.97, 129.96, 131.14, 132.42, 143.19, 144.35, 146.29, 158.98, 160.42, 168.97. MS (EI) m/z: 393.1 (M+); Anal. ($C_{23}H_{15}N_6O_2$) C, H, N.

4.1.7.2. 6-(2-(4-Cyanophenylamino)pyrimidin-4-yloxy)-7-methoxy-2-naphthonitrile (3b). Yield 41.8%; recrystallized from 1,4-dioxane, mp 253.2–254.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.83 (s, 3H, OCH_3), 6.69 (d, J = 5.6 Hz, 1H), 7.28–7.67 (m, 4H, Ph), 7.54–8.52 (m, 5H, naph), 8.45 (d, J = 5.6 Hz, 1H), 10.07 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 56.63, 99.94, 102.97, 108.98, 109.02, 118.66, 119.79, 119.81, 121.34, 125.39. 129.25, 130.47. 131.98, 132.98, 133.04, 144.47, 144.92, 152.33, 159.40. 160.67, 169.56. MS (EI) m/z: 393.1 (M+); Anal. ($C_{24}H_{17}N_5O_3$) C, H, N.

4.1.7.3. 6-(2-(4-Cyanophenylamino)pyrimidin-4-yloxy)-5,7-dimethoxy-2-naphthonitrile (3c). Yield 85.9%; flash chromatography separation and then recrystallized from 1,4-dioxane, 249.3–250.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.85 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 6.78 (d, J = 4.0 Hz, 1H), 7.24–7.52 (m, 4H, Ph), 7.54–8.54 (m, 4H, naph), 8.49 (d, J = 4.0 Hz, 1H), 10.11 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 56.83, 62.23, 99.77, 102.98, 104.10, 109.61, 118.62, 119.63, 119.76, 123.76, 125.20, 125.46, 131.61, 132.93, 133.28, 135.82, 144.87, 148.00, 153.19, 160.83, 169.18. MS (EI) m/z: 423.2 (M+); Anal. ($C_{23}H_{15}N_6O_2$) C, H, N.

4.1.7.4. 5-Chloro-6-(2-(4-cyanophenylamino)pyrimidin-4-yloxy)-2-naphthonitrile (3d). Yield 71.6%; recrystallized from 1,4-dioxane, mp 297.5–298.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.82 (d,

J = 4.0 Hz, 1H), 7.27–7.52 (m, 4H, Ph), 8.02–8.54 (m, 5H, naph), 8.82 (s, 1H, CH), 10.12 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 100.18, 103.15, 109.78, 118.81, 119.09, 119.72, 123.09, 125.27, 125.55, 129.19, 130.08, 131.49, 132.81, 132.93, 135.93, 144.72, 159.33, 161.18, 168.88. MS (EI) m/z: 393.1 (M+); Anal. ($C_{22}H_{12}ClN_5O$) C, H, N.

4.1.7.5. 5-Chloro-6-(2-(4-cyanophenylamino)pyrimidin-4-yloxy)-7-methoxy-2-naphthonitrile (3e). Yield 33.4%; flash chromatography separation and recrystallized from 1,4-dioxane, mp 268.4–269.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12 ^{13}C NMR (400 MHz, DMSO-d_6) δ 3.89 (s, 3H, CH_3), 6.82 (d, J = 4.4 Hz, 1H), 7.25–7..49 (m, 4H, Ph), 7.82–8.63 (m, 4H, naph), 8.52 (d, J = 4.4 Hz, 1H), 10.11 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 57.21, 99.71, 103.15, 108.27, 110.23, 118.66, 119.25, 119.72, 124.69, 125.54, 126.74, 127.57, 131.95, 132.94, 133.76, 141.45, 144.75, 152.66, 159.35, 161.13, 168.47. MS (EI) m/z: 427.1 (M+); Anal. ($C_{23}H_{14}ClN_5O_2$) C, H, N.

4.1.7.6. 6-(2-(4-Cyanophenylamino)-5-methylpyrimidin-4-yloxy)-2-naphthonitrile (3f). Yield 85.6%; recrystallized from 1,4-dioxane, mp 258.4–259.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.28 (s, 3H, CH_3), 7.27–7.57 (m, 4H, Ph), 7.65–8.40 (m, 4H, naph), 8.72(s, 1H, CH), 9.93 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.25, 102.46, 108.57, 118.40, 119.48, 119.64, 119.89, 124.61, 127.55, 129.40, 130.30, 130.95, 132.92, 134.79, 135.85, 145.16, 152.43, 157.75, 159.89, 167.90; MS (EI) m/z: 377.1 (M+); Anal. ($C_{23}H_{15}N_6BrO_2$) C, H, N.

4.1.7.7. 5-Bromo-6-(2-(4-cyanophenylamino)-5-methylpyrimidin-4-yloxy)-2-naphthonitrile (3g). Yield 83.3%; recrystallized from 1,4-dioxane, mp 268.4–269.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.43 (s, 3H, CH_3), 6.70 (s, 1H, CH), 7.23–7.52 (m, 4H, Ph), 7.76–8.82 (m, 5H, naph), 10.09 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 24.16, 98.81, 102.94, 109.70. 115.32, 118.69, 119.10, 119.77, 125.40, 128.19, 129.36, 130.78, 131.58, 132.89, 134.24, 135.47, 144.91, 151.15, 158.93, 169.35, 171.15; MS (EI) m/z: 457.0 (M+); Anal. ($C_{23}H_{15}N_5BrO_2$) C, H, N.

4.1.7.8. 6-(2-(4-Cyanophenylamino)-6-methylpyrimidin-4-yloxy)-2-naphthonitrile (3h). Yield 82.4%; recrystallized from 1,4-dioxane, mp 223.4–224.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.24 (s, 3H, CH_3), 7.24–7.54 (m, 4H, P), 7.62–8.68 (m, 6H, naph), 8.36 (s, 1H, CH), 9.90 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.25, 102.45, 108.57, 118.39 (2C), 119.48, 119.65, 119.89, 124.61, 127.54, 129.40, 130.29, 132.94 (2C), 134.78, 135.84, 145.15, 153.41, 157.73, 159.87, 167.89; MS (EI) m/z: 377.1 (M+); Anal. ($C_{23}H_{15}N_6O_2$) C, H, N.

4.1.7.9. 5-Bromo-6-(2-(4-cyanophenylamino)-6-methylpyrimidin-4-yloxy)-2-naphthonitrile (3i). Yield 81.8%; recrystallized from 1,4-dioxane, mp 254.4–225.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.30 (s, 3H, CH_3), 7.17–7.43 (m, 4H, Ph), 7.78–8.80 (m, 5H, naph), 8.40 (s, 1H, CH), 9.91 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.17, 102.57, 109.22, 109.69, 115.26, 118.33, 119.10, 119.81, 125.64, 128.17, 129.35, 130.70, 131.57, 132.86, 134.22, 135.46, 145.01, 151.42, 157.67, 160.17, 167.10; MS (EI) m/z: 457.0 (M+); Anal. ($C_{23}H_{15}N_6BrO_2$) C, H, N.

4.2. Biological study

4.2.1. Anti-HIV assay in MT-4 cells

The anti-HIV activity and cytotoxicity were evaluated against wild-type HIV-1 strain IIIB in MT-4 cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[11,12] Briefly, virus stocks were titrated in MT-4 cells and expressed as the 50% cell culture infective dose ($CCID_{50}$). MT-4 cells were suspended in culture medium at 1×10^5 cells/mL and infected with HIV at a multiplicity of infection of 0.02. Immediately after virus infection, 100 μL of the cell suspension was placed in each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. Stock solutions of the test compounds were dissolved in DMSO at 50 mM or higher. After 4 days of incubation of virus-infected cells with the compounds at 37 °C, the number of viable cells was determined using the MTT method. Compounds were tested in parallel for cytotoxic effects in uninfected MT-4 cells.

4.2.2. HIV RT assay

Reactions were performed under the conditions described for the HIV-1 RT RNA-dependent DNA polymerase activity assay. Incorporation of Biotin-dUTP into poly(rA)/oligo(dT) at different concentrations of DNA or Biotin-dUTP was monitored in the presence of increasing fixed amounts of inhibitors. The activity of RT was obtained through testing the amount of Biotin-dUTP by HRP-labeled Streptavidin. For IC_{50} determinations, a range of inhibitor concentrations between 0.2 IC_{50} and 5 IC_{50} was used.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (No. 20872018 and 30672536) and the Science and Technology Commission of Shanghai Municipality (No. 09431902800).

References and notes

1. Liang, Y. H.; Chen, F. E. *Eur. J. Med. Chem.* **2009**, *44*, 625.
2. Feng, X. Q.; Liang, Y. H.; Zeng, Z. S.; Chen, F. E.; Balzarini, J.; Pannecouque, C.; De Clercq, E. *ChemMedChem* **2009**, *4*, 219.
3. Liang, Y. H.; Feng, X. Q.; Zeng, Z. S.; Chen, F. E.; Balzarini, J.; Pannecouque, C.; De Clercq, E. *ChemMedChem* **2009**, *4*, 1537.
4. Ludovici, D. W.; De Corte, B. L.; Kukla, M. J.; Ye, H.; Ho, C. Y.; Lichtenstein, M. A.; Kavash, R. W.; Andries, K.; de Béthune, M. P.; Azijn, H.; Pauwels, R.; Lewi, P. J.; Heeres, J.; Koymans, L. M. H.; de Jonge, M. R.; Van Aken, K. J. A.; Daeyaert, F. F. D.; Das, K.; Arnold, E.; Janssen, P. A. J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2235.
5. Guillemont, J.; Pasquier, E.; Palandjian, P.; Vernier, D.; Gaurrand, S.; Lewi, P. J.; Heeres, J.; De Jonge, M. R.; Koymans, L. M. H.; Daeyaert, F. F. D.; Vinkers, M. H.; Arnold, E.; Das, K.; Pauwels, R.; Andries, K.; de Béthune, M. P.; Bettens, E.; Hertogs, K.; Wigerinck, P.; Timmerman, P.; Janssen, P. A. J. *J. Med. Chem.* **2005**, *48*, 2072.
6. Mordant, C.; Schmitt, B.; Pasquier, E.; Demestre, C.; Queguiner, L.; Masungi, C.; Peeters, A.; Smeulders, L.; Bettern, E.; Hertogs, K.; Heeres, J.; Lewi, P.; Guillemont, J. *Eur. J. Med. Chem.* **2007**, *42*, 567.
7. Haubrich, R.; Gubernick, S.; Yasothan, U.; Kirkpatrick, P. *Nat. Rev. Drug Disc.* **2008**, *7*, 287.
8. Frauzen, H. *J. Prakt. Chem.* **1921**, *103*, 369.
9. Fries, K.; Schimmelschmidt, K. *Lieb. Ann. Chem.* **1930**, *484*, 245.
10. Appendino, G.; Daddario, N.; Minassi, A.; Moriello, A. S.; De Petrocellis, L.; Di Marzo, V. *J. Med. Chem.* **2005**, *48*, 4663.
11. Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. *J. Virol. Methods* **1988**, *20*, 309.
12. Pannecouque, C.; Daelemans, D.; De Clercq, E. *Nat. Protocols* **2008**, *3*, 427.
13. Goodsell, D. S.; Morris, G. M.; Olson, A. J. *J. Mol. Recognit.* **1996**, *9*, 1.