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Structural Modifications of DAPY Analogues with Potent Anti-HIV-1 Activity

Xiao-Qing Feng,[a] Yong-Hong Liang,[a] Zhao-Sen Zeng,[a] Fen-Er Chen,*[a, b] Jan Balzarini,[c] Christophe Pannecouque,[c] and Erik De Clercq[c]

A novel series of diarylpyrimidine analogues (DAPYs) featuring a naphthyl moiety at the C4 position were synthesized and evaluated for their in vitro activity against HIV in MT-4 cells. All compounds exhibited strong activity against wild-type HIV-1. The most active compound showed activity against wild-type HIV-1 with an EC_{50} value of 2.35 nM and against the double mutant strain (K103N + Y181C) with an EC_{50} value of 6.6 μM, with a selectivity index greater than 60 000 against wild-type HIV-1. Additionally, some compounds also showed activity against HIV-2 (EC_{50} = 5.82 μM).

Introduction

Since they were first reported in 2001,[1] diarylpyrimidine derivatives (DAPYs), a novel class of non-nucleoside reverse transcriptase inhibitors (NNRTIs), have attracted considerable attention due to their excellent potency against wild-type and mutant strains of HIV-1 reverse transcriptase (HIV-1 RT), relative to other NNRTIs.[2–6] Further modifications of DAPYs have focused on wing I substituents and the pyrimidine scaffold, leading to the synthesis of many promising lead compounds[7–9] with strong potency against drug-resistant virus strains. Among these, etravirine (TMC125, **2**, Figure 1) has been approved by the U.S. Food and Drug Administration (FDA) for the treatment of patients infected with HIV-1 variants that are resistant to other anti-retroviral drugs.[10]

Our long-standing work on NNRTI pyrimidine analogues such as HEPTs (**3**),[11] DABOs (**4**),[12] DATAs (**5**)[13,14] and DAPYs[15] (Figure 1) has indicated that replacement of the phenyl ring at the C6 position of the pyrimidine ring with a bulky naphthyl moiety is beneficial by improving the π–π stacking interactions between inhibitors and amino acid residues Tyr 181, Tyr 188, and Trp 229 within the binding pocket of RT. Herein we report the synthesis, antiviral activity, and preliminary structure–activity relationships (SARs) of these new naphthyl-substituted DAPYs.

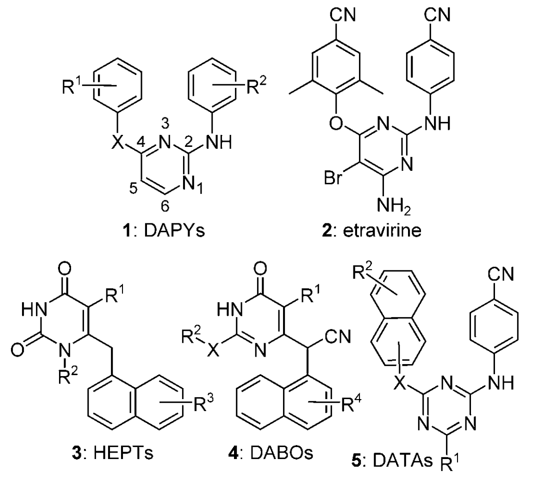

Figure 1. Chemical structures of DAPYs, HEPTs, DABOs, DATAs, and etravirine.

Results and Discussion

Chemistry

Target compounds **10a–af** in this study were synthesized as depicted in Scheme 1. Key intermediate 2-(methylthio)pyrimidin-4(1*H*)-ones **7a–c** were readily prepared by S-alkylation of thiouracils **6a–c** with iodomethane according to our previous reported protocol.[13] Compounds **7a–c** were condensed with 4-cyanoaniline at 180–190 °C for 8 hours under solvent-free conditions to afford 4-(4-oxo-1,4-dihydropyrimidin-2-ylamino)-benzonitriles **8a–c**.[16] Subsequent treatment with $POCl_3$ at reflux for 30 minutes provided the corresponding 4-(4-chloropyrimidin-2-ylamino)benzonitriles **9a–c**. Treatment of **9a–c** with appropriate naphthol or naphthiol derivatives in the presence of K_2CO_3 in anhydrous DMF at 110 °C under nitrogen atmosphere gave desired compounds **10a–af** in yields ranging from 20.1 to 87.9%.

[a] X.-Q. Feng, Y.-H. Liang, Z.-S. Zeng, Prof. Dr. F.-E. Chen
Department of Chemistry, Fudan University
Shanghai 200433 (P.R. China)
Fax: (+86) 21-6564 3811
E-mail: rfchen@fudan.edu.cn

[b] Prof. Dr. F.-E. Chen
Institute of Biomedical Science, Fudan University
Shanghai 200433 (P.R. China)

[c] Prof. Dr. J. Balzarini, Prof. Dr. C. Pannecouque, Prof. Dr. E. De Clercq
Rega Institute for Medical Research
Katholieke Universiteit Leuven
10 Minderbroedersstraat, 3000 Leuven (Belgium)

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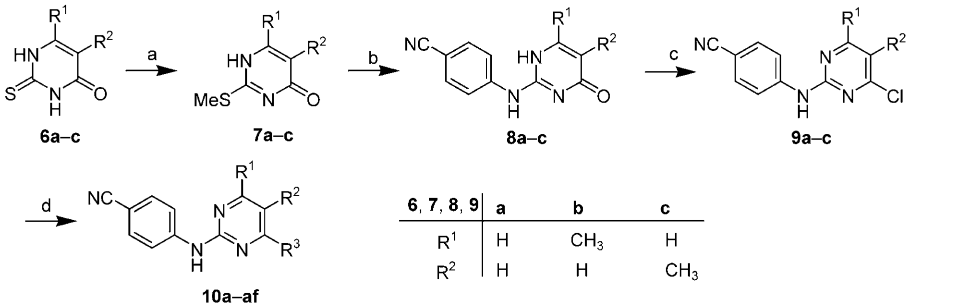

6, 7, 8, 9	a	b	c
R1	H	CH_3	H
R2	H	H	CH_3

10	R1	R2	R3	10	R1	R2	R3	10	R1	R2	R3
a	Me	H	1-naphthoxy	l	H	Me	2-naphthoxy	w	H	H	1-Br-2-naphthoxy
b	H	Me	1-naphthoxy	m	H	H	2-naphthoxy	x	Me	H	1,6-diBr-2-naphthoxy
c	H	H	1-naphthoxy	n	Me	H	2-naphththio	y	H	Me	1,6-diBr-2-naphthoxy
d	H	H	1-naphththio	o	H	Me	2-naphththio	z	H	H	1,6-diBr-2-naphthoxy
e	Me	H	4-Cl-1-naphthoxy	p	H	H	2-naphththio	aa	Me	H	6-Br-2-naphthoxy
f	H	Me	4-Cl-1-naphthoxy	q	H	Br	2-naphththio	ab	H	Me	6-Br-2-naphthoxy
g	H	H	4-Cl-1-naphthoxy	r	Me	H	1-Cl-2-naphthoxy	ac	H	H	6-Br-2-naphthoxy
h	Me	H	4-Br-1-naphthoxy	s	H	Me	1-Cl-2-naphthoxy	ad	Me	H	phenoxy
i	H	Me	4-Br-1-naphthoxy	t	H	H	1-Cl-2-naphthoxy	ae	H	Me	phenoxy
j	H	H	4-Br-1-naphthoxy	u	Me	H	1-Br-2-naphthoxy	af	H	H	phenoxy
k	Me	H	2-naphthoxy	v	H	Me	1-Br-2-naphthoxy				

Scheme 1. Synthetic route to compounds **10a–af**. Reagents and conditions: a) NaOH, room temperature, 30 min, then MeI, room temperature, 24 h; b) 4-cyanoaniline, 180–190 °C, ~8 h; c) $POCl_3$, reflux, 30 min; d) potassium carbonate, naphthol or naphthiol derivative, DMF, 5 min, then **9**, 110 °C, N_2, 8–12 h.

Biological activity

The MTT method[17,18] was used to evaluate 29 new naphthyl-substituted compounds, **10a**–**10ac**, and 3-phenylsubstituted compounds, **10ad**–**10af**, along with three FDA-approved drugs for reference purposes: nevirapine (NEV), delavirdine (DEV), and efavirenz (EFV). These compounds were all assessed for their cytotoxicity and antiviral activities in MT-4 cells infected with the HIV-1 wild-type virus (LAI strain, IIIB), HIV-1 double mutant virus (K103N+Y181C, with Lys 103 replaced by Asn and Tyr 181 by Cys), or HIV-2 strain ROD.

Two series of DAPY derivatives: 1-naphthyl-substituted compounds **10a**–**j** and 2-naphthyl-substituted compounds **10k**–**ac**, were generally very active against wild-type HIV-1 and did not exhibit cytotoxicity up to 24.86 μM, with the exception of **10b**, as shown in Table 1. The anti-retroviral activity (EC_{50}) ranged from 2.35 nM (**10w**) to 111.53 nM (**10aa**); the selectivity index (SI) ranged from 309 (**10h**) to >106 232 (**10t**). The 1-naphthyl- and 2-naphthyl-substituted parent compounds **10c**–**d**, **10m**, and **10p** were more potent than NEV and DEV, and demonstrated inhibitory potency in the low-nanomolar range with high SI values. With the aim of validating our design rationale, three compounds, **10ad**–**af**, incorporating a phenyl ring at the C4 position of the pyrimidine ring were synthesized. These compounds exhibited poor activity against the HIV-1 LAI virus, and were 3- to 12-fold less potent than the naphthyl-substituted compounds **10c**–**d**, **10m**, and **10p**. These results confirmed our initial hypothesis that replacement of the phenyl moiety with a naphthyl moiety improves the putative π–π stacking interactions between inhibitors and amino acid residues Tyr 181, Tyr 188, and Trp 229 within the binding pocket of RT.

To investigate structural variations of the pyrimidine and naphthalene rings, a series of DAPYs with substitutions at the C4 position of these rings was synthesized and evaluated for antiviral activity. Introduction of a methyl group on the pyrimidine ring for the 1-naphthyl or 2-naphthyl series resulted in negligible loss of anti-HIV-1 (wild-type) activity. On the other hand, it is interesting to note that **10b**, **10f**, and **10i**, with methyl substituents at the C5 position, were more potent than the corresponding nonsubstituted compounds in the 1-naphthyl series. However, in the 2-napthyl series, **10m**, **10w**, and **10z**, which lack substituents at the C5 position, exhibited the highest potency. Introduction of a chlorine or bromine at the *para* position (**10e**–**j**) resulted in minor changes in activity as compared with **10a**–**c** for the 1-naphthyl series. Conversely, in the 2-naphthyl series, the 1-chloro- or bromo-substituted compounds **10r**–**w** showed significant improvements in potency compared with original hits **10k**–**m**, with **10w** displaying the greatest activity (EC_{50} = 2.35 nM).

To investigate the potency of these compounds against drug-resistant virus, the activities against the double mutant strain K103N+Y181C were also evaluated. The compounds with chlorine or bromine substituents at the C1 position in the 2-naphthyl series exhibited low-micromolar antiviral activity

Table 1. Anti-HIV-1 activity and cytotoxicity of compounds **10a–af** in MT-4 cells.

Compd	EC_{50} [nM][a] wild-type (IIIB)	EC_{50} [μM][a] HIV-2	EC_{50} [μM][a] K103N+Y181C	CC_{50} [μM][b]	SI[c]
10a	68.96±36.04	>34.56	>34.56	34.56±1.79	501
10b	3.35±0.45	>4.06	>4.06	4.06±1.53	1220
10c	21.87±13.39	>369.43	>369.43	>369.43	>16903
10d	5.87±3.72	16.17	>252.18	252.18±24.55	43072
10e	60.49±12.41	>58.22	>58.22	58.22±18.04	964
10f	9.33±1.78	>323.14	>323.14	>323.14	>34626
10g	12.15±0.08	>335.29	>335.29	>335.29	>27594
10h	80.46±30.84	>24.86	>24.86	24.86±1.18	309
10i	10.39±1.07	>212.53	>212.53	212.53±26.32	20445
10j	23.97±6.95	>203.71	>203.71	≥203.71	≥8492
10k	54.77±1.14	≥14.02	>156.3	156.3±61.32	2861
10l	9.96±1.48	>354.72	>354.72	>354.72	>35663
10m	9.78±2.19	223.14	>369.43	>369.43	>37764
10n	47.22±9.50	7.06	>30.32	30.32±5.75	642
10o	24.4±15.14	>251.87	>251.87	≥251.87	≥10323
10p	12.70±1.41	≥10.13	>47.20	47.20±2.82	3754
10q	49.16±51.23	18.35	>129.77	129.77±82.59	2636
10r	11.40±0.75	6.08	4.27	77.35±53.46	6790
10s	3.83±1.22	>323.14	7.55	>323.14	>84650
10t	3.17±0.40	168.77	52.84	>335.29	>106232
10u	8.42±2.67	5.82	4.52	38.95±7.23	4634
10v	4.57±0.60	6.72	9.51	>289.83	>63613
10w	2.35±0.07	15.94	6.57	153.5±10.40	65591
10x	18.42±13.33	≥50.18	≥213.65	≥213.65	≥11602
10y	12.96±6.47	75.07	>245.01	>245.01	>18925
10z	11.67±3.06	136.15	>251.94	>251.94	>21608
10aa	111.53±57.50	>230.82	>230.82	230.82±42.78	2070
10ab	85.56±32.00	>289.83	>289.83	>289.83	>3391
10ac	25.4±22.29	>299.57	>299.57	>299.57	>11767
10ad	262.3±202.10	>21.00	>21.00	21±14.12	80
10ae	37.71±20.18	>0.30	>0.30	0.3±0.05	8
10af	70.76±28.79	>433.58	>433.58	>433.58	>6137
NEV	75.1	–	–	>15.02	>252
EFV	3	–	–	>6.336	>2174
DEV	72	–	–	>3.619	44

[a] EC_{50}: compound concentration required to protect the cell against viral cytopathogenicity by 50% in MT-4 cells. [b] CC_{50}: compound concentration that decreases the normal uninfected MT-4 cell viability by 50%. [c] SI: selectivity index; ratio CC_{50}/EC_{50} (wild-type).

against the double mutant strain: (**10s**, $EC_{50}=7.55$ μM; **10r**, $EC_{50}=4.27$ μM; **10u**, $EC_{50}=4.52$ μM; **10v**, $EC_{50}=9.51$ μM; and **10w**, $EC_{50}=6.57$ μM). These promising results promote further investigation of this new series of NNRTIs.

All title compounds were also assessed for their abilities to inhibit replication of the HIV-2 ROD virus in MT-4 cells, as depicted in Table 1. Some compounds also displayed activity against HIV-2 at micromolar concentrations, particularly **10n** ($EC_{50}=7.06$ μM), **10r** ($EC_{50}=6.08$ μM), **10u** ($EC_{50}=5.82$ μM), **10v** ($EC_{50}=6.72$ μM), and **10w** ($EC_{50}=15.94$ μM); however, these compounds were also fairly cytotoxic to the host cells.

Molecular modeling analysis

To investigate the possible binding conformations of our newly synthesized compounds and their interaction mode with RT, a modeling study was performed using the AutoDock 4.0.1 program.[19] Compound **10m** was chosen to be docked into the non-nucleoside inhibitor binding pocket (NNIBP) of HIV-1 RT. Coordinates of the NNIBP were taken from a crystal structure of the RT–TMC278 complex, owing to the high degree of similarity between TMC278 and **10m**.[20] The theoretical binding mode of **10m** to the NNIBP is shown in Figure 2. In comparison with the binding of TMC125 to RT,[21] the naphthyl-substituted DAPY **10m** enhanced π–π and π–H interactions with amino acid residues Tyr 181, Tyr 188, and Phe 227, over those of the original cyanovinyl group in TMC278.[20] These binding data support our initial design; however, these compounds lack the strong interactions with Trp 229 that play an important role in the activity of TMC278 and TMC125 against drug-resistant mutant strains.

Conclusions

In summary, we designed and synthesized a novel series of naphthyl-substituted DAPYs. Biological test results indicated that the designed compounds showed potent antiviral activity, with EC_{50} values in the low-nanomolar range, and did not exhibit cytotoxicity up to 24 860 nM, with the exception of **10b**. The 1-bromo-2-naphthoxy compound **10w** was the most potent inhibitor of wild-type HIV-1 ($EC_{50}=2.35$ nM in MT-4 cells); however, the activity of **10w** against the double mutant

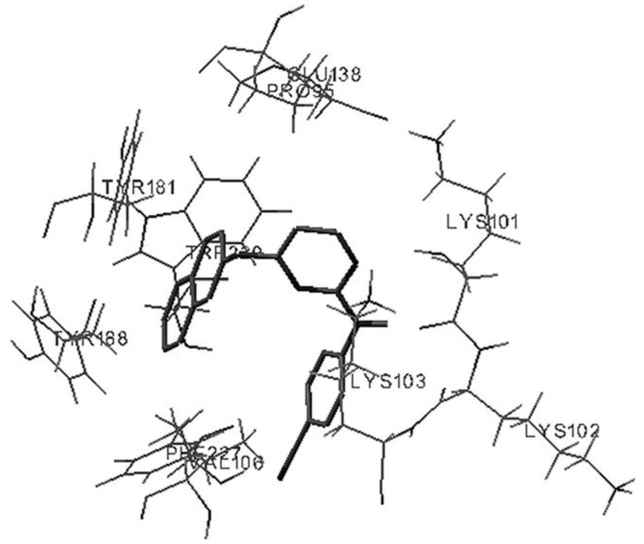

Figure 2. Model of **10m** docked within the non-nucleoside binding site of RT.

strain K103N+Y181C were still at the micromolar level. These results serve to support further modification of the naphthalene ring in an attempt to improve activity against drug-resistant mutant strains.

Experimental Section

Chemistry. Melting points were measured on a WRS-1 digital melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded in [D$_6$]DMSO using a Bruker AV 400 MHz spectrometer. Chemical shifts are reported in δ (ppm) units relative to the internal standard tetramethylsilane (TMS). Mass spectra were obtained on an Agilent MS/5975 mass spectrometer. Elemental analyses were performed on a Carlo Erba 1106 instrument, and the results of elemental analyses for C, H, Cl, N, and S were within ±0.4% of 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 reactions were monitored by TLC on pre-coated silica gel G plates at 254 nm under a UV lamp using EtOAc/hexanes as eluents. Flash chromatography separations were obtained using silica gel (300–400 mesh).

General procedure for the synthesis of 7a–c. NaOH (8.0 g, 300 mmol) was added portion-wise to a suspension of thiouracils **6a–c** (300 mmol) in H_2O at room temperature. After the reaction mixture was stirred for 30 min, iodomethane (320 mmol) was added, and stirring continued at room temperature for 24 h. The precipitate was filtered off, washed with H_2O, and dried to give 2-(methylthio)pyrimidin-4(1*H*)-ones **6a–c** to be used without further purification.

General procedure for the synthesis of 8a–c. 2-(methylthio)pyrimidin-4(1*H*)-ones **7a–c** (200 mmol) and 4-cyanoaniline (500 mmol) were thoroughly mixed. The mixture was slowly heated to 180–190 °C and maintained at this temperature until no odor of methanethiol was perceptible (about 8 h).[15] After cooling and dissolving the mixture in EtOH, and subsequent decolorization with charcoal, the product was precipitated with H_2O. The suspension was acidified (pH 3) with HCl to dissolve remaining starting materials. The precipitate was filtered and washed with H_2O. Final products were crystallized from 90% EtOH.

General procedure for the synthesis of 9a–c. A mixture of 40 mL $POCl_3$ and intermediate 4-(4-oxo-1,4-dihydropyrimidin-2-ylamino)-benzonitriles **8a–c** (150 mmol) was held at reflux for 30 min. The mixture was poured into 250 mL ice-water and stirred at room temperature for 3 h. The resulting precipitate was filtered, washed with 50 mL H_2O, and dried to give 4-(4-chloropyrimidin-2-ylamino)-benzonitriles **9a–c** to be used without further purification.

General procedure for the synthesis of 10a–af. Potassium carbonate (10 mmol) was added to a solution of naphthol or naphthiol derivatives (2 mmol) in 20 mL anhydrous DMF and stirred for 5 min. One of the 4-(4-chloropyrimidin-2-ylamino)benzonitriles **9a–c** (2 mmol) was then added. The mixture was heated at 110 °C under nitrogen atmosphere for 8–12 h. Next, the mixture was treated with cold H_2O (200 mL), and the resulting precipitate was collected by filtration. Crude products **10a–af** were recrystallized from toluene.

4-(4-methyl-6-(naphthalen-1-yloxy)pyrimidin-2-ylamino)benzonitrile (10a). Yield 23.2%; recrystallized from toluene, mp: 142.8–143.8 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ=2.39 (s, 3H, CH_3), 6.62 (s, 1H, CH), 7.31–7.41 (m, 4H, Ph), 7.43–8.10 (m, 7H, Naph), 10.06 ppm (s, 1H, NH); MS (EI) *m/z*: 352 [*M*]$^+$; Anal. ($C_{22}H_{16}N_4O$) C, H, N.

4-(5-methyl-4-(naphthalen-1-yloxy)pyrimidin-2-ylamino)benzonitrile (10b). Yield 23.9%; recrystallized from toluene, mp: 178.6–181.5 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ=2.37 (s, 3H, CH_3), 7.24–7.29 (m, 4H, Ph), 7.43–8.10 (m, 7H, Naph), 8.38 (s, 1H, CH), 9.86 ppm (s, 1H, NH); MS (EI) *m/z*: 352 [*M*]$^+$; Anal. ($C_{22}H_{16}N_4O$) C, H, N.

4-(4-(naphthalen-1-yloxy)pyrimidin-2-ylamino)benzonitrile (10c). Yield 22.1%; recrystallized from toluene, mp: 205.7–207.4 °C; ^{1}H NMR (DMSO, 400 MHz) δ=6.75 (d, 1H, *J*=6.0 Hz, CH), 7.34–7.42 (m, 4H, Ph), 7.44–8.01 (m, 7H, Naph), 8.49 (d, 1H, *J*=6.0 Hz, CH), 10.09 ppm (s, 1H, NH); MS (EI) *m/z*: 338 [*M*]$^+$; Anal. ($C_{21}H_{14}N_4O$) C, H, N.

4-(4-(naphthalen-1-ylthio)pyrimidin-2-ylamino)benzonitrile (10d). Yield 35.2%; recrystallized from toluene, mp: 208.0–208.6 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ=6.52 (d, 1H, *J*=6.0 Hz, CH), 7.34 (s, 4H, Ph), 7.56–8.28 (m, 7H, Naph), 8.19 (d, 1H, *J*=4.4 Hz, CH), 10.16 ppm (s, 1H, NH); MS (EI) *m/z*: 354 [*M*]$^+$; Anal. ($C_{21}H_{14}N_4S$) C, H, N, S.

4-(4-(4-chloronaphthalen-1-yloxy)-6-methylpyrimidin-2-ylamino)-benzonitrile (10e). Yield 27.3%; recrystallized from toluene, mp: 195.7–197.0 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ=2.39 (s, 3H, CH_3), 6.65 (s, 1H, CH), 7.33–7.43 (m, 4H, Ph), 7.45–8.28 (m, 6H, Naph), 10.06 ppm (s, 1H, NH); MS (EI) *m/z*: 386 [*M*]$^+$; Anal. ($C_{22}H_{15}N_4OCl$) C, H, N, Cl.

4-(4-(4-chloronaphthalen-1-yloxy)-5-methylpyrimidin-2-ylamino)-benzonitrile (10f). Yield 31.0%; recrystallized from toluene, mp: 247.2–247.9 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ=2.36 (s, 3H, CH_3), 7.26–7.33 (m, 4H, Ph), 7.46–8.30 (m, 6H, Naph), 8.39 (s, 1H, CH), 9.87 ppm (s, 1H, NH); MS (EI) *m/z*: 386 [*M*]$^+$; Anal. ($C_{22}H_{15}N_4OCl$) C, H, N, Cl.

4-(4-(4-chloronaphthalen-1-yloxy)pyrimidin-2-ylamino)benzonitrile (10g). Yield 47.1%; recrystallized from toluene, mp: 220.8–221.6 °C; ^{1}H NMR (DMSO, 400 MHz) δ=6.79 (d, 1H, *J*=6.4 Hz, CH), 7.37–7.45 (m, 4H, Ph), 7.47–8.29 (m, 6H, Naph), 8.50 (d, 1H, *J*=

6.4 Hz, CH), 10.08 ppm (s, 1H, NH); MS (EI) *m/z*: 373 $[M]^+$; Anal. ($C_{21}H_{13}N_4OCl$) C, H, N, Cl.

4-(4-(4-bromonaphthalen-1-yloxy)-6-methylpyrimidin-2-ylamino)benzonitrile (10h). Yield 20.6%; recrystallized from toluene, mp: 193.4–195.3 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ = 2.41 (s, 3H, CH_3), 6.67 (s, 1H, CH), 7.33–7.41 (m, 4H, Ph), 7.62–8.25 (m, 6H, Naph), 10.05 ppm (s, 1H, NH); MS (EI) *m/z*: 431$[M]^+$; Anal. ($C_{22}H_{15}N_4OBr$) C, H, N, Br.

4-(4-(4-bromonaphthalen-1-yloxy)-5-methylpyrimidin-2-ylamino)benzonitrile (10i). Yield 25.3%; recrystallized from toluene, mp 248.9–250.7 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ = 2.34 (s, 3H, CH_3), 7.26–7.30 (m, 4H, Ph), 7.38–8.24 (m, 6H, Naph), 8.37 (s, 1H, CH), 9.84 ppm (s, 1H, NH); MS (EI) *m/z* 431 $[M]^+$; Anal. ($C_{22}H_{15}N_4OBr$) C, H, N, Br.

4-(4-(4-bromonaphthalen-1-yloxy)pyrimidin-2-ylamino)benzonitrile (10j). Yield 87.9%; recrystallized from toluene mp: 208.9–209.8 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ = 6.79 (d, 1H, J = 6.4 Hz, CH), 7.36–7.44 (m, 4H, Ph), 7.62–8.25 (m, 6H, Naph), 8.50 (d, 1H, J = 6.4 Hz, CH), 10.06 ppm (s, 1H, NH); MS (EI) *m/z*: 417 $[M]^+$; Anal. ($C_{21}H_{13}N_4OBr$) C, H, N, Br.

4-(4-methyl-6-(naphthalen-2-yloxy)pyrimidin-2-ylamino)benzonitrile (10k). Yield 44.4%; recrystallized from toluene, mp: 168.8–170.8 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ = 2.38 (s, 3H, CH3), 6.52 (s, 1H, CH), 7.25–7.64 (m, 4H, Ph), 7.41–8.08 (m, 7H, Naph), 10.10 ppm (s, 1H, NH); MS (EI) *m/z*: 352 $[M]^+$; Anal. ($C_{22}H_{16}N_4O$) C, H, N.

4-(5-methyl-4-(naphthalen-2-yloxy)pyrimidin-2-ylamino)benzonitrile (10l). Yield 21.9%; recrystallized from toluene, mp: 209.9–211.3 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ = 2.26 (s, 3H, CH3), 7.15–7.53 (m, 4H, Ph), 7.42–8.08 (m, 7H, Naph), 8.34 (s, 1H, CH), 9.91 ppm (s, 1H, NH); MS (EI) *m/z* 352 $[M]^+$; Anal. ($C_{22}H_{16}N_4O$) C, H, N.

4-(4-(naphthalen-2-yloxy)pyrimidin-2-ylamino)benzonitrile (10m). Yield 30.5%; recrystallized from toluene, mp: 204.7–206.4 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ = 6.70 (d, 1H, J = 6.0 Hz, CH), 7.31–7.68 (m, 4H, Ph), 7.47–8.12 (m, 7H, Naph), 8.50 (d, 1H, J = 6.0 Hz, CH), 10.16 ppm (s, 1H, NH); MS (EI) *m/z*: 338 $[M]^+$; Anal. ($C_{21}H_{14}N_4O$) C, H, N.

4-(4-methyl-6-(naphthalen-2-ylthio)pyrimidin-2-ylamino)benzonitrile (10n). Yield 41.8%; recrystallized from toluene, mp: 180.7–181.6 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ = 2.37 (s, 3H, CH3), 6.66 (s, 1H, CH), 6.94–7.41 (m, 4H, Ph), 7.63–8.31 (m, 7H, Naph), 10.11 ppm (s, 1H, NH); MS (EI) *m/z*: 368 $[M]^+$; Anal. ($C_{22}H_{16}N_4S$) C, H, N, S.

4-(5-methyl-4-(naphthalen-2-ylthio)pyrimidin-2-ylamino)benzonitrile (10o). Yield 21.4%; recrystallized from toluene, mp: 214.3–215.7 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ = 2.27 (s, 3H, CH_3), 6.47–7.05 (m, 4H, Ph), 7.60–8.18 (m, 7H, Naph), 8.30 (s, 1H, CH), 9.91 ppm (s, 1H, NH); MS (EI) *m/z*: 368 $[M]^+$; Anal. ($C_{22}H_{16}N_4S$) C, H, N, S.

4-(4-(naphthalen-2-ylthio)pyrimidin-2-ylamino)benzonitrile (10p). Yield 27.5%; recrystallized from toluene, mp: 183.0–183.3 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ = 6.70 (d„ 1H, J = 5.6 Hz, CH), 7.00–7.45 (m, 4H, Ph), 7.62–8.26 (m, 7H, Naph), 8.33 (s, 1H, CH), 10.18 ppm (s, 1H, NH); MS (EI) *m/z*: 354 $[M]^+$; Anal. ($C_{21}H_{14}N_4S$) C, H, N, S.

4-(5-bromo-4-(naphthalen-2-ylthio)pyrimidin-2-ylamino)benzonitrile (10q). Yield 32.1%; recrystallized from toluene, mp: 193.2–194.1 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ = 6.46–6.98 (m, 4H, Ph), 7.64–8.35 (m, 7H, Naph), 8.48 (s, 1H, CH), 10.20 ppm (s, 1H, NH); MS (EI) *m/z*: 433 $[M]^+$; Anal. ($C_{21}H_{13}N_4SBr$) C, H, N, Br.

4-(4-(1-chloronaphthalen-2-yloxy)-6-methylpyrimidin-2-ylamino)benzonitrile (10r). Yield 20.7%; recrystallized from toluene, mp 222.3–224 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ = 2.42 (s, 3H, CH_3), 6.67 (s, 1H, CH), 7.11–7.48 (m, 4H, Ph), 7.58–8.23 (m, 6H, Naph), 10.10 ppm (s, 1H, NH); MS (EI) *m/z*: 386 $[M]^+$; Anal. ($C_{22}H_{15}N_4OCl$) C, H, N, Cl.

4-(4-(1-chloronaphthalen-2-yloxy)-5-methylpyrimidin-2-ylamino)benzonitrile (10s). Yield 39.3%; recrystallized from EtOAc, mp: 225.7–227.4 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ = 2.30 (s, 3H, CH_3), 7.06–7.43 (m, 4H, Ph), 7.59–8.21 (m, 6H, Naph), 8.37 (s, 1H, CH), 9.94 ppm (s, 1H, NH); MS (EI) *m/z*: 406 $[M]^+$; Anal. ($C_{22}H_{15}N_4OCl$) C, H, N, Cl.

4-(4-(1-chloronaphthalen-2-yloxy)pyrimidin-2-ylamino)benzonitrile (10t). Yield 20.1%; recrystallized from toluene, mp: 201.9–203.2 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ = 6.79 (d, 1H, J = 5.6 Hz, CH), 7.15–7.51 (m, 4H, Ph), 7.60–8.23 (m, 6H, Naph), 8.52 (d, 1H, J = 5.6 Hz, CH), 10.15 ppm (s, 1H, NH); MS (EI) *m/z*: 372 $[M]^+$; Anal. ($C_{21}H_{13}N_4OCl$) C, H, N, Cl.

4-(4-(1-bromonaphthalen-2-yloxy)-6-methylpyrimidin-2-ylamino)benzonitrile (10u). Yield 44.5%; recrystallized from toluene, mp: 216.4–217.5 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ = 2.41 (s, 3H, CH_3), 6.65 (s, 1H, CH), 7.12–7.49 (m, 4H, Ph), 7.55–8.21 (m, 6H, Naph), 10.11 ppm (s, 1H, NH); MS (EI) *m/z*: 431 $[M]^+$; Anal. ($C_{22}H_{15}N_4OBr$) C, H, N, Br.

4-(4-(1-bromonaphthalen-2-yloxy)-5-methylpyrimidin-2-ylamino)benzonitrile (10v). Yield 35.9%; recrystallized from MeOH, mp: 233.7–236.1 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ = 2.31 (s, 3H, CH_3), 7.07–7.42 (m, 4H, Ph), 7.59–8.21 (m, 6H, Naph), 8.39 (s, 1H, CH), 9.94 ppm (s, 1H, NH); MS (EI) *m/z*: 431 $[M]^+$; Anal. ($C_{22}H_{15}N_4OBr$) C, H, N, Br.

4-(4-(1-bromonaphthalen-2-yloxy)pyrimidin-2-ylamino)benzonitrile (10w). Yield 33.0%; recrystallized from toluene, mp: 187.6–189.9 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ = 6.78 (d, 1H, J = 5.6 Hz, CH), 7.17–7.52 (m, 4H, Ph), 7.58–8.21 (m, 6H, Naph), 8.52 (d, 1H, J = 5.6 Hz, CH), 10.13 ppm (s, 1H, NH); MS (EI) *m/z*:417 $[M]^+$; Anal. ($C_{21}H_{13}N_4OBr$) C, H, N, Br.

4-(4-(1,6-dibromonaphthalen-2-yloxy)-6-methylpyrimidin-2-ylamino)benzonitrile (10x). Yield 53.6%; recrystallized from toluene, mp: 243.2–244.2 °C; ^{1}H NMR (DMSO, 400 MHz) δ = 2.40 (s, 3H, CH_3), 6.64 (s, 1H, CH), 7.19–7.50 (m, 4H, Ph), 7.48–8.44 (m, 5H, Naph), 10.09 ppm (s, 1H, NH); MS (EI) *m/z*: 510 $[M]^+$; Anal. ($C_{22}H_{14}N_4OBr_2$) C, H, N, Br.

4-(4-(1,6-dibromonaphthalen-2-yloxy)-5-methylpyrimidin-2-ylamino)benzonitrile (10y). Yield 37.6%; recrystallized from toluene, mp: 240.8–242.1 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ = 2.29 (s, 3H, CH_3), 7.16–7.44 (m, 4H, Ph), 7.64–8.38 (m, 5H, Naph), 8.46 (s, 1H, CH), 9.94 ppm (s, 1H, NH); MS (EI) *m/z*: 510 $[M]^+$; Anal. ($C_{22}H_{14}N_4OBr_2$) C, H, N, Br.

4-(4-(1,6-dibromonaphthalen-2-yloxy)pyrimidin-2-ylamino)benzonitrile (10z). Yield 29.9%; recrystallized from toluene, mp: 231.4–232.1 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ = 6.70 (d, 1H, J = 5.6 Hz, CH), 7.24–7.54 (m, 4H, Ph), 7.64–8.46 (m, 5H, Naph), 8.52 (d, 1H, J = 5.6 Hz, CH), 10.11 ppm (s, 1H, NH); MS (EI) *m/z*: 496 $[M]^+$; Anal. ($C_{21}H_{12}N_4OBr_2$) C, H, N, Br.

4-(4-(6-bromonaphthalen-2-yloxy)-6-methylpyrimidin-2-ylamino)benzonitrile (10aa). Yield 22.5%; recrystallized from toluene, mp: 200.8–201.4 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ=2.29 (s, 3H, CH_3), 6.63 (s, 1H, CH), 7.31–7.65 (m, 4H, Ph), 7.48–8.33 (m, 6H, Naph), 10.09 ppm (s, 1H, NH); MS (EI) *m/z*: 431 [*M*]$^+$; Anal. ($C_{22}H_{15}N_4OBr$) C, H, N, Br.

4-(4-(6-bromonaphthalen-2-yloxy)-5-methylpyrimidin-2-ylamino)benzonitrile (10ab). Yield 26.1%; recrystallized from toluene, mp 239.3–240.3 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ=2.25 (s, 3H, CH_3), 7.23–7.53 (m, 4H, Ph), 7.55–8.08 (m, 6H, Naph), 8.35 (s, 1H, CH), 9.91 ppm (s, 1H, NH); MS (EI) *m/z*: 431 [*M*]$^+$; Anal. ($C_{22}H_{15}N_4OBr$) C, H, N, Br.

4-(4-(6-bromonaphthalen-2-yloxy)pyrimidin-2-ylamino)benzonitrile (10ac). Yield 23.7%; recrystallized from toluene, mp: 234.2–236.2 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ=6.67 (d, 1H, *J*=5.6 Hz, CH), 7.34–7.66 (m, 4H, Ph), 7.51–8.34 (m, 6H, Naph), 8.48 (d, 1H, *J*=5.6 Hz, CH), 10.11 ppm (s, 1H, NH); MS (EI) *m/z*: 417 [*M*]$^+$; Anal. ($C_{21}H_{13}N_4OBr$) C, H, N, Br.

4-(4-methyl-6-phenoxypyrimidin-2-ylamino)benzonitrile (10ad). Yield 20.4%; recrystallized from toluene, mp: 138.4–141.4 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ=2.35 (s, 3H, CH_3), 6.43 (s, 1H, CH), 7.22–7.67 (m, 4H, Ph), 7.35–7.52 (m, 5H, Ph), 10.09 ppm (s, 1H, NH); MS (EI) *m/z*: 302 [*M*]$^+$; Anal. ($C_{18}H_{14}N_4O$) C, H, N.

4-(5-methyl-4-phenoxypyrimidin-2-ylamino)benzonitrile (10ae). Yield 20.3%; recrystallized from toluene, mp: 170.0–171.5 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ=2.20 (s, 3H, CH_3), 7.23–7.58 (m, 4H, Ph), 7.36–7.53 (m, 5H, Ph), 8.29 (s, 1H, CH), 9.94 ppm (s, 1H, NH); MS (EI) *m/z*: 302 [*M*]$^+$; Anal. ($C_{18}H_{14}N_4O$) C, H, N.

4-(4-phenoxypyrimidin-2-ylamino)benzonitrile (10af). Yield 28.7%; recrystallized from toluene, mp: 188.6–189.7 °C; ^{1}H NMR ([D$_6$]DMSO, 400 MHz) δ=6.67 (d, 1H, *J*=5.6 Hz, CH), 7.25–7.69 (m, 4H, Ph), 7.35–7.53(m, 5H, Ph), 8.43 (d, 1H, *J*=5.6 Hz, CH), 10.13 ppm (s, 1H, NH); MS (EI) *m/z*: 288 [*M*]$^+$; Anal. ($C_{17}H_{12}N_4O$) C, H, N.

Evaluation method. 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.[17,18] 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^{-1} and infected with HIV at a multiplicity of infection of 0.02. Immediately after viral 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 these compounds were dissolved in DMSO at 50 mM or higher. After incubation of virus-infected cells with the compounds at 37 °C for 4 days, the number of viable cells was determined using the MTT method. Compounds were tested in parallel for cytotoxic effects in uninfected MT-4 cells.

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