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

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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 EC₅₀ of 0.16 and 0.15 μ M, and were more activity than that of efavirenz.

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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 naph-thyl-substituted DAPY analogues¹⁻³ 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.

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Scheme 1. Reagents and conditions: (a) K₂CO₃, DMF, 110 °C, 8-12 h, N₂.

was synthesized from 6-cyano-2-naphthol (**4**). Then, the treatment of the intermediate **5** with Br₂ 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 phosphory-lated for NRTI activity against RT. As shown in Table 1, the 6-cya-no-2-naphthyl substituted DAPY derivatives displayed strong inhibitory activity against wild-type HIV-1 at nanomolar concentration level (EC₅₀ = 0.012–0.002 μ M) and low cytotoxicity (CC₅₀ = 118.44–331.56 μ M), 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 μ M), 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 **3i**.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 **31**.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 EC₅₀ value of 0.005 and 0.007 μ M, 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 dualsubstituted compound with RT, a molecular docking study was performed using the program AUTODOCK 4.0.1¹³ 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) Br₂, AcOH, AcONa, rt, 2 h; (ii) SnCl₂(H₂O)₂, AcOH, 5 h; (c) MeONa, Cul, DMF, 110 °C, 3 h; (d) Sn, HCl, AcOH, reflux, 4 h; (e) Br₂, 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 ₅	EC_{50}^{b} (μ M)		SI ^d
						WT(IIIB)	103 N+181C		
3a	Н	Н	OMe	Н	0.9	0.002	0.38	>211.45	≥134,032
3b	Н	Н	Н	OMe	0.52	0.002	318.07	>318.07	>158,228
3c	Н	Н	OMe	OMe	0.04	0.005	0.16	>118.44	≥25,000
3d	Н	Н	Cl	Н	nd	0.006	0.70	268.79	47,964
3e	Н	Н	Cl	OMe	nd	0.007	0.15	>190.65	≥25,701
3f	Me	Н	Н	Н	nd	0.007	331.56	>331.56	>44,856
3g	Me	Н	Br	Н	nd	0.012	0.83	>274.12	>23,779
3h	Н	Me	Н	Н	nd	0.003	331.56	>331.56	>96,451
3i	Н	Me	Br	Н	nd	0.007	0.22	>274.12	>40,068
3j ³	Н	Н	Н	Н	3.17	0.003	6.30	>68.80	20,548
3k ³	Н	Н	Br	Н	1.33	0.002	0.24	282.63	181,247
31 ³	Н	Н	Н	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 CC₅₀/EC₅₀ (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 μ M 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. ¹H NMR and ¹³C 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 airsensitive 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; ¹H 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 SnCl₂(H₂O)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; ¹H 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; ¹H 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; ¹H NMR (400 MHz, DMSO-*d*₆) δ 3.85 (s, 3H, OCH₃), 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 ¹H NMR (400 MHz, DMSO-*d*₆) δ 3.85 (s, 3H, CH₃), 3.93 (s, 3H, CH₃), 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; ¹H NMR (400 MHz, DMSO- d_6) δ 3.91 (s, 3H, CH₃), 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; ¹H 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; ¹H NMR (400 MHz, DMSO- d_6) δ 3.98 (s, 3H, CH₃), 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; ¹H NMR (400 MHz, DMSO- d_6) δ 3.90 (s, 3H, CH₃), 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); ¹³C 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. (C₂₃H₁₅N₆O₂) C, H, N.

4.1.7.2. 6-(2-(4-Cyanophenylamino)pyrimidin-4-yloxy)-7-methoxy-2-naphthonitrile (3b). Yield 41.8%; recrystallized from 1,4dioxane, mp 253.2–254.8 °C; ¹H NMR (400 MHz, DMSO- d_6) δ 3.83 (s, 3H, OCH₃), 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); ¹³C 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. (C₂₄H₁₇N₅O₃) 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; ¹H NMR (400 MHz, DMSO- d_6) δ 3.85 (s, 3H, OCH₃), 3.91 (s, 3H, OCH₃), 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); ¹³C 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. (C₂₃H₁₅N₆O₂) 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; ¹H 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); ¹³C 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. (C₂₂H₁₂ClN₅O) 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; ¹H NMR (400 MHz, DMSO- d_6) δ 12 ¹³C NMR (400 MHz, DMSO- d_6) δ 3.89 (s, 3H, CH₃), 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); ¹³C 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. (C₂₃H₁₄ClN₅O₂) 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; ¹H NMR (400 MHz, DMSO- d_6) δ 2.28 (s, 3H, CH₃), 7.27–7.57 (m, 4H, Ph), 7.65–8.40 (m, 4H, naph), 8.72(s, 1H, CH), 9.93 (s, 1H, NH); ¹³C 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. (C₂₃H₁₅N₆BrO₂) 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; ¹H NMR (400 MHz, DMSO d_6) δ 2.43 (s, 3H, CH₃), 6.70 (s, 1H, CH), 7.23–7.52 (m, 4H, Ph), 7.76–8.82 (m, 5H, naph), 10.09 (s, 1H, NH); ¹³C 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. (C₂₃H₁₅N₅BrO₂) 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; ¹H NMR (400 MHz, DMSO- d_6) δ 2.24 (s, 3H, CH₃), 7.24–7.54 (m, 4H, P), 7.62–8.68 (m, 6H, naph), 8.36 (s, 1H, CH), 9.90 (s, 1H, NH); ¹³C 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. (C₂₃H₁₅N₆O₂) 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; ¹H NMR (400 MHz, DMSO d_6) δ 2.30 (s, 3H, CH₃), 7.17–7.43 (m, 4H, Ph), 7.78–8.80 (m, 5H, naph), 8.40 (s, 1H, CH), 9.91 (s, 1H, NH); ¹³C 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. (C₂₃H₁₅N₆BrO₂) 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 (CCID₅₀). 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 HRPlabeled Streptavidin. For IC₅₀ determinations, a range of inhibitor concentrations between 0.2 IC₅₀ and 5 IC₅₀ was used.

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