



Synthesis and structure–activity relationship of novel diarylpyrimidines with hydromethyl linker ($CH(OH)$ -DAPYs) as HIV-1 NNRTIs

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ABSTRACT

A series of 26 diarylpyrimidines, characterized by the hydroxymethyl linker between the left wing benzene ring and the central pyrimidine, were synthesized and evaluated for in vitro anti-HIV activity. Most of the compounds exhibited moderate to excellent activities against wild-type HIV-1. Among them, compound **10i**, bearing a chlorine atom at the C-2 position of left benzene ring, was the best congener and showed potent activity against wild-type HIV-1 with an EC_{50} value of 0.009 μ M, along with moderate activities against the double RT mutant (K103N + Y181C) HIV-1(III_B) and HIV-2(ROD) with an EC_{50} value of 6.2 and 6.0 μ M, respectively. The preliminary structure–activity relationship (SAR) of this new series of compounds was also investigated.

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1. Introduction

Since 1996, highly active antiretroviral therapy (HAART) has been adopted as an efficient way to treat AIDS patients by reducing viral loads and restoring the immune system.¹ Nonnucleoside reverse transcriptase inhibitors (NNRTIs), known as one of the indispensable components of HAART for specifically inhibiting HIV-1 reverse transcriptase (RT), have been paid wide attention due to their unique antiviral potency, high specificity and low cytotoxicity.²

With the aim to develop novel anti-HIV drugs, various structurally different classes of NNRTIs, such as benzophenones,³ diaryl ethers,⁴ 1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio)thymine (HEPTs),^{5,6} dihydro-alkoxybenzyl-oxypyrimidines (DABOs),⁷ diaryl-triazines (DATAs),⁸ dihydro-aryl/alkylsulfanyl-cyclohexyl-methyloxy-pyrimidines (S-DACOs)⁹ and diaryl-pyrimidines (DAPYs),¹⁰ have been discovered. Among these series, DAPYs have been recognized as one of the most successful family of NNRTIs developed so far due to their excellent potency against HIV-1 wild-type (WT) and mutant strains.¹¹ In the DAPY series, etravirine (TMC125, **1**, Fig. 1)^{10a,12} has been approved by the US Food and Drug Administration (FDA) in 2008, and rilpivirine (TMC278, **2**, Fig. 1)¹³ has been regarded as another promising anti-HIV drug that has recently also been approved. Since the right wing of the DAPY structure was

confirmed as the indispensable pharmacophore, the further modifications were mainly focused on the structural diversity of the linker between the left benzene ring and the central pyrimidine ring.^{2b} In terms of different linkers, DAPYs contain a variety of molecules (Fig. 1) as follows: CH_2 -DAPYs (**3**),^{10a} O -DAPYs (**4**),^{10a,b,f,i} S -DAPYs (**5**),^{10a} NH -DAPYs (**6**),^{10a,13} $C(=NOH)$ -DAPYs (**7**),^{10e} $CH(CN)$ -DAPYs (**8**)^{10g} and $CH(Me)$ -DAPYs (**9**).^{10j} Based on these different structures, we conceived that the introduction of a hydrophilic hydroxyl group to the CH_2 linker of **3** could offer a structurally novel scaffold for further development of a new series of NNRTIs (**10**, $CH(OH)$ -DAPYs). The hydroxymethyl linker would make the molecules retain good molecular flexibility, which might help the compounds to adopt multiple conformations within the RT nonnucleoside binding site.^{10j,14} In the present work, the synthesis and the structure–activity relationship (SAR) of the series of $CH(OH)$ -DAPYs are described.

2. Results and discussion

2.1. Chemistry

The synthesis of the target compounds **10a–z** were shown in Scheme 1. The key intermediates 4-((4-chloropyrimidin-2-yl)amino)benzonitriles (**14a–c**) were prepared from **11a–c** through the modified methods of our previously reported 3-step synthetic strategy.^{10f,g} Treatment of **14a–c** with the corresponding phenyl-acetonitriles in the presence of 60% NaH in anhydrous DMF at room

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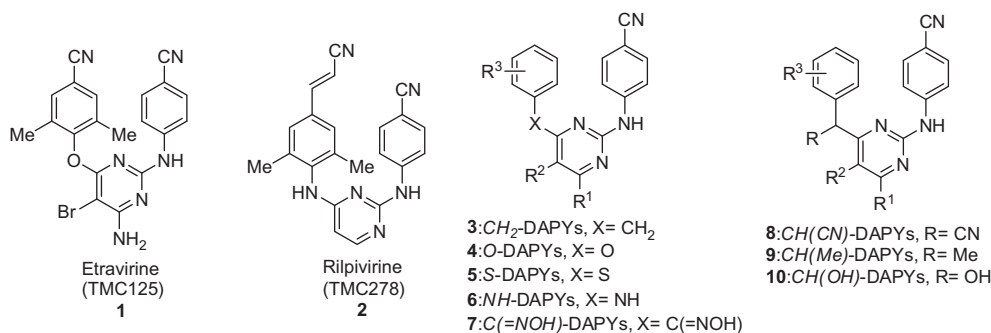
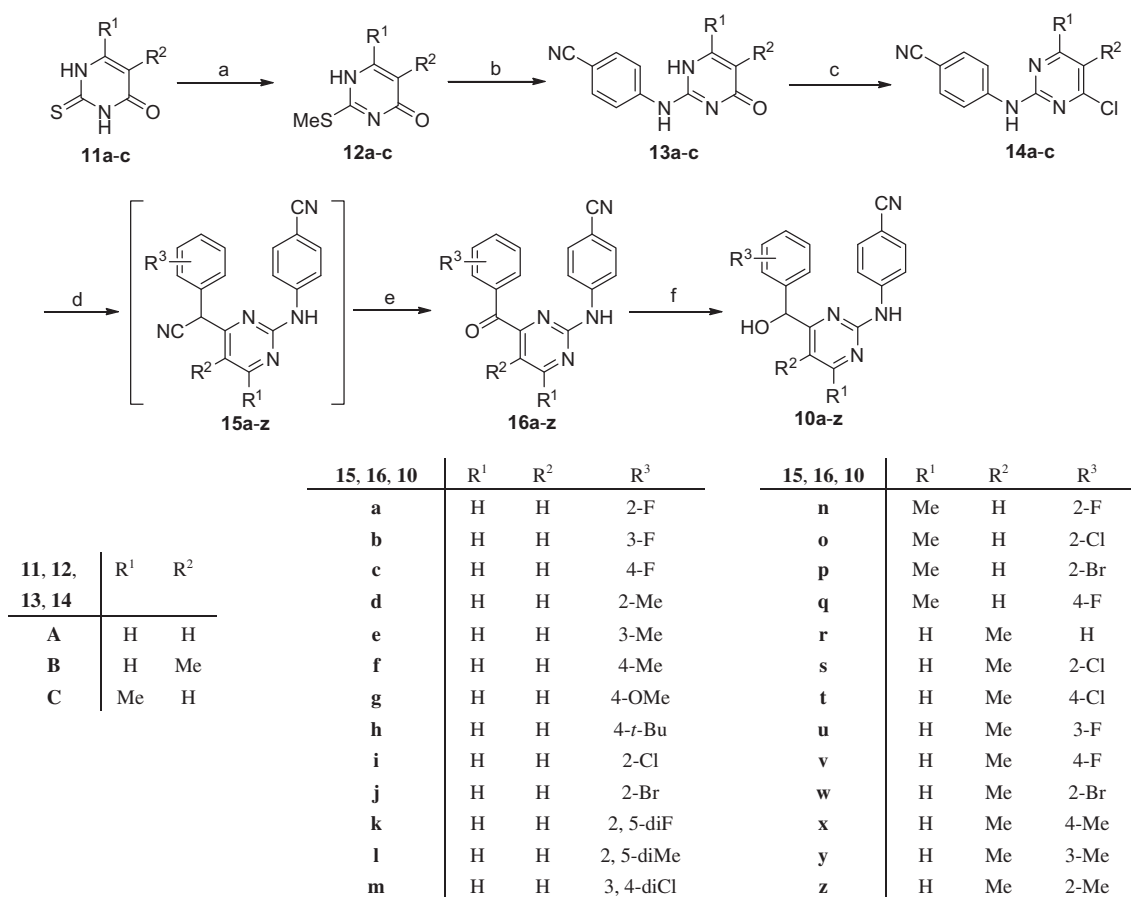


Figure 1. Structures of DAPYs.



Scheme 1. Synthetic route to CH(OH)-DAPYs (**10a–z**). Reagents and conditions: (a) MeI, NaOH, H₂O, rt, 24 h; (b) 4-cyanoaniline, 180–190 °C, 10 h; (c) POCl₃, reflux, 0.5 h; (d) R₃-phenylacetonitrile, 60% NaH, Ar, DMF, –20 °C to rt, 48–72 h; (e) NaH, air, DMF, rt, 36–72 h; (f) NaBH₄, MeOH, 0 °C to rt, 2 h.

temperature for 48–72 h under argon atmosphere gave 4-(4-(cyanoaryl)methyl)pyrimidin-2-ylamino)benzonitriles (**15a–z**), which were subsequently subjected to natural oxidation by exposing the reaction mixture to air at room temperature under stirring conditions for 36–72 h to afford the 4-(4-benzoylpyrimidin-2-ylamino)-benzonitriles (**16a–z**).^{10e} Reductions of **16a–z** with potassium borohydride in methanol provided the title compounds 4-((4-(hydroxy(phenyl)methyl)pyrimidin-2-yl)amino)benzonitriles (**10a–z**) in yields of 64.8–90.9%.

2.2. Biological activity

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) method¹⁵ was used to evaluate 26 new CH(OH)-DAPYs

(**10a–z**) along with five FDA-approved drugs: nevirapine, zidovudine, zalcitabine, delavirdine, and efavirenz as reference compounds. These compounds were assayed for their cytotoxicity and anti-HIV activities in MT-4 cells infected with wild-type HIV-1 (LAI strain, III_B), double RT mutant (K103N + Y181C) HIV-1, with Lys 103 replaced by Asn and Tyr181 by Cys, and HIV-2 strain ROD. The results, expressed as CC₅₀ (50% cytotoxic concentration), EC₅₀ (50% HIV-1 replication inhibitory concentration) and SI (selectivity index given by the CC₅₀/EC₅₀ ratio) values, are summarized in Table 1.

As listed in Table 1, most CH(OH)-DAPYs showed moderate to potent activities against wild-type (WT) HIV-1 with EC₅₀ values in the range of 9.870–0.008 μM. In particular, the compound **10i** displayed excellent anti-HIV-1 activity against WT HIV-1 with an

Table 1
Anti-HIV activities and cytotoxicity of compounds **10a–z** in MT-4 cells^a

Compd	EC ₅₀ (μM) ^b			CC ₅₀ (μM) ^c	SI ^d
	WT (III _B)	K103N + Y181C	HIV-2		
10a	0.031 ± 0.013	8.1 ± 1.8	8.1 ± 0.9	38.6 ± 1.2	1271
10b	0.562 ± 0.343	>34.7	>34.7	34.7 ± 5.7	61
10c	5.931 ± 3.996	>252.6	>252.6	252.6 ± 43.5	43
10d	0.047 ± 0.016	>38.6	>38.6	38.6 ± 3.3	822
10e	0.348 ± 0.253	>38.4	>38.4	38.4 ± 2.9	115
10f	0.070 ± 0.025	>41.8	>41.8	41.8 ± 1.7	597
10g	0.220 ± 0.145	>151.0	>151.0	151.0 ± 64.4	687
10h	0.335 ± 0.084	>35.3	>35.3	35.5 ± 3.2	110
10i	0.009 ± 0.003	6.2 ± 0.3	6.0 ± 0.2	35.8 ± 5.0	4115
10j	0.009 ± 0.006	10.2 ± 0.3	≥ 11.4	34.2 ± 3.4	3924
10k	0.047 ± 0.027	>7.6	>7.6	7.6 ± 0.9	164
10l	0.010 ± 0.010	>36.6	>36.6	36.6 ± 5.5	3600
10m	0.008 ± 0.001	>1.8	>1.8	1.8 ± 0.1	223
10n	0.123 ± 0.111	>21.4	>21.4	21.4 ± 3.3	175
10o	0.029 ± 0.011	>31.6	>31.6	31.6 ± 2.9	1075
10p	0.149 ± 0.243	>27.0	>27.0	27.0 ± 1.9	180
10q	9.870 ± 3.440	>34.6	>34.6	34.6 ± 3.7	4
10r	7.302 ± 7.049	>80.5	>80.5	80.5 ± 56.0	11
10s	0.051 ± 0.014	>21.1	>21.1	21.1 ± 6.2	408
10t	7.925 ± 1.767	>40.4	>40.4	40.4 ± 15.2	5
10u	1.140 ± 0.428	>36.4	>36.4	36.4 ± 2.3	32
10v	>37.177	–	>37.2	37.2 ± 2.2	<1
10w	5.516 ± 0.835	>30.3	>30.3	30.3 ± 2.7	5
10x	2.815 ± 1.544	>32.9	>32.9	32.9 ± 1.3	12
10y	1.029 ± 0.575	>378.4	>378.4	>378.4	>364
10z	0.333 ± 0.121	>41.4	>41.4	41.4 ± 33.1	130
Nevirapine	0.180 ± 0.083	>9.50	–	>15.02	>83
Zidovudine	0.007 ± 0.001	0.006 ± 0.002	0.006 ± 0.001	>93.55	>13799
Zalcitabine	1.468 ± 0.237	–	1.56 ± 0.14	>94.69	>65
Efavirenz	0.005 ± 0.001	0.443 ± 0.063	–	>6.33	>1146
Delavirdine	0.160 ± 0.112	>4.380	–	>4.38	>27
Etravirine	0.003 ± 0.001	0.026 ± 0.010	–	>4.6	>1537

^a Data represent the mean of at least three separate experiments.

^b Compound concentration required to protect MT-4 cells against viral cytopathogenicity by 50%.

^c Compound concentration that decreases the uninfected MT-4 cell viability by 50%.

^d Selectivity index: CC₅₀/EC₅₀ ratio (WT).

EC₅₀ value of 0.009 μM and the greatest selectivity (SI = 4115), along with moderate activities against the double RT mutant virus (K103N + Y181C, EC₅₀ = 6.2 μM) and HIV-2 (EC₅₀ = 6.0 μM). Apparently, it is more potent than nevirapine, zalcitabine, and delavirdine in inhibiting WT HIV-1. In addition, the other three compounds (**10j**, **10l**, **10m**) also displayed the anti-HIV-1 WT activities ranging from 0.010 to 0.008 μM, which are also proximal to that of the best compound **10i**.

To explore the structure–activity relationship (SAR) of the new CH(OH)-DAPYs, the modifications are focused on the left wing as well as the central pyrimidine ring. In general, the methyl group at the C-5 or -6 position on the pyrimidine ring affords great harm to the anti-HIV-1 (WT) activity of CH(OH)-DAPYs, and the former one seems to be more unfavorable. When there is no substituent on the pyrimidine ring, most of the compounds (**10a–m**) displayed potent activities with EC₅₀ values lower than 0.1 μM, especially, four of them (**10i**, **10j** and **10l**, **10m**) are the most potent compounds among these analogs.

For the compounds with an unsubstituted pyrimidine and a mono-substituent at the C-2 position of the left wing, the electron-withdrawing halogens seem to be more advantageous than the electron-donating alkyl and alkoxy groups. The activity of the compounds with halogens at the benzene ring displayed following order of activity: *ortho*-F (**10a**) > *meta*-F (**10b**) > *para*-F (**10c**), *ortho*-F (**10a**) < *ortho*-Cl (**10i**) = *ortho*-Br (**10j**). For electro-donating groups, the activity sequence is *ortho*-Me (**10d**) > *para*-Me (**10f**) > *meta*-Me (**10e**), and the bulky group appeared to be more disadvantageous: 4-*t*-Bu (**10h**) < 4-OMe (**10g**) < 4-Me (**10f**). When the disubstituted halogens are installed on the benzene ring, the

resulting compounds also showed potent activities against WT HIV-1. However, the di-halogenated analogs showed higher toxicity and low inhibitory selectivity. Typically, the compound **10m** displayed the most potent activity against WT HIV-1 (EC₅₀ = 0.008 μM) but at a low selectivity index (SI = 223).

Among the analogs **10n–q** with a methyl group at the C-6 position of the pyrimidine, the chlorine in the *ortho*-position (**10o**) also proved to be the most favorable for the activity (EC₅₀ = 0.029 μM) and selectivity (SI = 1075), and the fluorine in the *para*-position on the benzene ring (**10q**) is still intolerable in comparison with the *ortho*-position fluorine (**10n**). Moreover, the compounds **10r–z**, with a methyl at the C-5 position of the pyrimidine, showed moderate to poor activity except the relatively potent *ortho*-chlorine analog (**10s**). When compared with the compound with no substituent on the left benzene ring (**10r**), the *ortho*- and *meta*-halogen could enhance the activities and the *para*-halogen would result in loss of activity. The introduction of *para*-fluorine (**10v**) led to the complete loss of activities against WT HIV-1 (EC₅₀ > 37 μM, SI < 1). The introduction of a methyl group is all favorable for the anti-HIV-1 WT activity with an obvious priority of *ortho*- > *meta*- > *para*-.

To investigate the potency against drug-resistant virus, these compounds were evaluated for their activity against the double RT mutant (K103N + Y181C) HIV-1 strain which was cross-resistant to most of currently available NNRTIs.^{3b,16} The compounds with an *ortho*-halogen on the left wing and no substituent on the dipyrimidine displayed moderate activities against the double mutant strain at a similar level: (**10a**, EC₅₀ = 8.1 μM; **10i**, EC₅₀ = 6.2 μM; **10j**, EC₅₀ = 10.2 μM).

All the *CH(OH)*-DAPYs were also assayed for their activities against HIV-2 (ROD) in MT-4 cells. As depicted in Table 1, some compounds also showed activities against HIV-2 at a micromolar level such as compounds **10a** (EC_{50} = 8.1 μ M) and **10i** (EC_{50} = 6.0 μ M). Although HIV-1 and HIV-2 reverse transcriptases are similar in structure and functionality,¹⁷ most of previously discovered NNRTIs are only active against HIV-1 and lack of anti-HIV-2 activity.^{4–9} Some of the synthesized *CH(OH)*-DAPYs displayed moderate anti-HIV-2 activities, whereas it is still difficult to develop NNRTIs that exhibit both anti-HIV-1 and -2 activities.

Compared with the most promising *C(=NOH)*-DAPY,^{10e} the most potent *CH(OH)*-DAPY compound **10i** has better activities against HIV-1 WT, the double RT mutant (K103N + Y181C) and HIV-2 ROD, which may be attributed to that the single bond CH–OH of **10i** determines its better molecular flexibility. Although the activities of some *CH(CN)*-DAPYs (**8**)^{10g} are more potent than *CH(OH)*-DAPYs, the activities of the former against the double RT mutant (K103N + Y181C) and HIV-2 ROD are strikingly weak than the latter.

Taking together the activities against HIV-1 WT, the double RT mutant (K103N + Y181C) HIV-1 strain and HIV-2 ROD, the compound **10i** is undoubtedly the most potent of all the synthesized *CH(OH)*-DAPYs.

3. Conclusion

In summary, we designed and synthesized a novel series of *CH(OH)*-DAPYs. Biological evaluation indicated that most of the designed compounds showed moderate to excellent anti-HIV activity. Among these compounds, the most active compound **10i**, which possess a chlorine atom at the C-2 position of the left benzene ring, showed potent activity against wild-type HIV-1 with an EC_{50} value of 0.009 μ M, along with moderate activities against the double RT mutant (K103N + Y181C) HIV-1 strain and HIV-2 strain ROD with EC_{50} values of 6.2 and 6.0 μ M, respectively. These results serve to support further modification of diarylpyrimidines in an attempt to search for more effective anti-HIV-1 candidates. As our continuing work, we would prepare the optical isomers of them by chemical resolution or asymmetric synthesis and assess their anti-HIV activities.

4. Experimental

4.1. Chemistry

Melting points were measured on a SGW X-1 microscopic 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 Waters Quattro Micromass instrument using electrospray ionization (ESI) techniques. Elemental analyses were performed on a Carlo Erba 1106 instrument. 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 thin layer chromatography (TLC) on pre-coated silica gel G plates at 254 nm under a UV lamp using ethyl acetate/petroleum ether as eluent. Flash chromatography separations were obtained on silica gel (300–400 mesh).

4.1.1. General procedure for the synthesis of 12a–c

NaOH (6.18 g, 155 mmol) was added portion-wise to a suspension of thiouracils **11a–c** (150 mmol) in H₂O at room temperature. After the reaction mixture was stirred for 30 min and cooled to 10 °C, iodomethane (25.55 g, 180 mmol) was added. The mixture was stirred for another 24 h at room temperature, followed by cooling to 5–10 °C. The precipitate was filtered off, washed with

H₂O, and dried to give 2-(methylthio)pyrimidin-4(1*H*)-ones **12a–c** to be used without further purification.

4.1.2. General procedure for the synthesis of 13a–c

The mixture of 2-(methylthio)-pyrimidin-4(1*H*)-ones **12a–c** (100 mmol) and 4-cyanoaniline (35.4 g, 300 mmol) was slowly heated to 180–190 °C and maintained at this temperature for 8 h. After cooling, the hard mixture was crushed by ultrasound treatment in CH₃CN (150 mL). Then the solid was filtered off and washed with CH₃CN until there was no residue 4-cyanoaniline detected by TLC.

4.1.3. General procedure for the synthesis of 14a–c

A mixture of POCl₃ (30 mL) and intermediate 4-(4-oxo-1,4-dihydropyrimidin-2-ylamino) benzonitriles **13a–c** (80 mmol) was stirred at reflux for 30 min. Then the mixture was poured into ice-water (250 mL) and stirred for 30 min at room temperature. The resulting precipitate was filtered, washed with H₂O to pH 6–7, and dried to give 4-(4-chloropyrimidin-2-ylamino)-benzonitriles **14a–c** to be used without further purification.

4.1.4. General procedure for the synthesis of 16a–z

A solution of **14a–c** (2 mmol), appropriate aryl acetonitrile (3 mmol) and anhydrous DMF (30 mL) was stirred for 0.5 h at –20 °C. Then NaH (0.14 g, 3.5 mmol; 60% dispersion in mineral oil) was added portionwise at –20 °C under Ar. The mixture was stirred at RT for 48–72 h under Ar, and then stirred under air for another 36–72 h at RT. The resulting mixture was poured into water and extracted with EtOAc. The combined organic layers were dried by Na₂SO₄, filtered and concentrated in vacuo. Purification by flash chromatography (silica gel; EtOAc/petroleum ether, 1:6 to 1:4) gave **16a–z**.

4.1.5. General procedure for the synthesis of target compounds 10a–z

KBH₄ (19 mg, 0.35 mol) was added to the mixture of **16a–z** (0.5 mmol) and methanol (15 mL) at 0–5 °C. The mixture was stirred for 2 h, then poured into water and extracted with EtOAc. The combined organic layers were dried by Na₂SO₄, filtered and concentrated in vacuo. Purification by flash chromatography (silica gel; EtOAc/petroleum ether, 1:5 to 1:3) gave **10a–z**.

4.1.5.1. 4-((4-((2-fluorophenyl)(hydroxy)methyl)pyrimidin-2-yl)amino)-benzonitrile (10a). Yield 81.7%; light yellow solid; mp 154.1–155.8 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 5.87 (d, *J* = 4.8 Hz, 1H, CH), 6.40 (d, *J* = 4.8 Hz, 1H, deuterium-exchanged, OH), 7.19–7.85 (m, 5H, pyrimidine H₅ + Ar'H), 7.62 (d, *J* = 8.8 Hz, 2H, ArH_{2,6}), 7.84 (d, *J* = 8.8 Hz, 2H, ArH_{3,5}), 8.59 (d, *J* = 5.2 Hz, 1H, pyrimidine H₆), 10.12 (s, 1H, NH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 68.78 (d, *J*_{CF} = 2.1 Hz), 102.27, 109.96, 115.30 (d, *J*_{CF} = 21.5 Hz), 118.18, 119.60, 124.49 (d, *J*_{CF} = 3.4 Hz), 129.02 (d, *J*_{CF} = 4.4 Hz), 129.53 (d, *J*_{CF} = 7.9 Hz), 130.28 (d, *J*_{CF} = 14.1 Hz), 132.82, 144.93, 158.83, 158.93, 159.70 (d, *J*_{CF} = 243.6 Hz), 172.54; MS (ESI+) *m/z* 321 (M+H)⁺; Anal. Calcd for C₁₈H₁₃FN₄O: C, 67.49; H, 4.09; F, 5.93; N, 17.49. Found: C, 67.42; H, 4.12; F, 5.96; N, 17.45.

4.1.5.2. 4-((4-((3-fluorophenyl)(hydroxy)methyl)pyrimidin-2-yl)amino)-benzonitrile (10b). Yield 83.9%; light pink solid; mp 149.5–150.8 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 5.63 (d, *J* = 4.0 Hz, 1H, CH), 6.41 (d, *J* = 4.0 Hz, 1H, deuterium-exchanged, OH), 7.07–7.43 (m, 5H, pyrimidine H₅ + Ar'H), 7.67 (d, *J* = 8.4 Hz, 2H, ArH_{2,6}), 7.92 (d, *J* = 8.4 Hz, 2H, ArH_{3,5}), 8.56 (d, *J* = 5.2 Hz, 1H, pyrimidine H₆), 10.16 (s, 1H, NH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 74.19, 102.32, 109.58, 113.34 (d, *J*_{CF} = 22.0 Hz), 114.18 (d, *J*_{CF} = 21.5 Hz), 118.28, 119.59, 122.85 (d, *J*_{CF} = 2.4 Hz), 130.19 (d, *J*_{CF} = 8.1 Hz), 132.88, 144.91, 145.99 (d, *J*_{CF} = 6.9 Hz), 158.77,

158.94, 162.13 (d, J_{CF} = 241.8 Hz), 172.88; MS (ESI+) m/z 321 (M+H)⁺; Anal. Calcd for C₁₈H₁₃FN₄O: C, 67.49; H, 4.09; F, 5.93; N, 17.49. Found: C, 67.52; H, 4.06; F, 5.89; N, 17.53.

4.1.5.3. 4-((4-((4-fluorophenyl)(hydroxy)methyl)pyrimidin-2-yl)amino)-benzonitrile (10c). Yield 77.8%; light yellow solid; mp 153.5–155.2 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 5.61 (d, J = 4.0 Hz, 1H, CH), 6.32 (d, J = 4.0 Hz, 1H, OH), 7.16–7.50 (m, 5H, pyrimidine H₅ + Ar'H), 7.68 (d, J = 8.4 Hz, 2H, ArH_{2,6}), 7.91 (d, J = 8.8 Hz, 2H, ArH_{3,5}), 8.56 (d, J = 5.2 Hz, 1H, pyrimidine H₆), 10.15 (s, 1H, NH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 74.19, 102.28, 109.48, 114.99 (d, J_{CF} = 21.2 Hz), 118.26, 119.62, 128.76 (d, J_{CF} = 7.8 Hz), 132.93, 139.36 (d, J_{CF} = 3.4 Hz), 144.94, 158.77, 158.90, 161.52 (d, J_{CF} = 241.7 Hz), 173.33; MS (ESI+) m/z 321 (M+H)⁺; Anal. Calcd for C₁₈H₁₃FN₄O: C, 67.49; H, 4.09; F, 5.93; N, 17.49. Found: C, 67.38; H, 4.13; F, 5.97; N, 17.46.

4.1.5.4. 4-((4-(hydroxy(o-tolyl)methyl)pyrimidin-2-yl)amino)-benzonitrile (10d). Yield 84.6%; light yellow solid; mp 177.5–179.1 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 2.38 (s, 3H, CH₃), 5.79 (d, J = 4.0 Hz, 1H, CH), 6.14 (d, J = 4.4 Hz, 1H, OH), 7.18–7.34 (m, 5H, pyrimidine H₅ + Ar'H), 7.62 (d, J = 8.8 Hz, 2H, ArH_{2,6}), 7.82 (d, J = 8.8 Hz, 2H, ArH_{3,5}), 8.56 (d, J = 5.2 Hz, 1H, pyrimidine H₆), 10.06 (s, 1H, NH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 19.29, 72.02, 102.17, 110.09, 118.17, 119.55, 125.80, 127.25, 127.28, 130.15, 132.77, 135.50, 141.46, 144.91, 158.66, 158.69, 173.48; MS (ESI+) m/z 317 (M+H)⁺; Anal. Calcd for C₁₉H₁₆N₄O: C, 72.13; H, 5.10; N, 17.71. Found: C, 72.08; H, 5.13; N, 17.66.

4.1.5.5. 4-((4-(hydroxy(m-tolyl)methyl)pyrimidin-2-yl)amino)-benzonitrile (10e). Yield 80.5%; light yellow solid; mp 129.4–130.7 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 2.29 (s, 3H, CH₃), 5.53 (d, J = 4.4 Hz, 1H, CH), 6.19 (d, J = 4.0 Hz, 1H, OH), 7.07–7.26 (m, 5H, pyrimidine H₅ + Ar'H), 7.67 (d, J = 8.8 Hz, 2H, ArH_{2,6}), 7.82 (d, J = 8.8 Hz, 2H, ArH_{3,5}), 8.56 (d, J = 5.2 Hz, 1H, pyrimidine H₆), 10.13 (s, 1H, NH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 21.04, 74.96, 102.19, 109.49, 118.20, 119.56, 123.92, 127.24, 127.99, 128.08, 132.86, 137.25, 143.01, 144.94, 158.66, 158.69, 173.60; MS (ESI+) m/z 317 (M+H)⁺; Anal. Calcd for C₁₉H₁₆N₄O: C, 72.13; H, 5.10; N, 17.71. Found: C, 72.18; H, 5.06; N, 17.73.

4.1.5.6. 4-((4-(hydroxy(p-tolyl)methyl)pyrimidin-2-yl)amino)-benzonitrile (10f). Yield 82.1%; light yellow solid; mp 154.8–156.9 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 2.26 (s, 3H, CH₃), 5.54 (d, 1H, J = 4.0 Hz, CH), 6.18 (d, 1H, J = 4.0 Hz, OH), 7.14–7.33 (m, 5H, pyrimidine H₅ + Ar'H), 7.68 (d, J = 8.8 Hz, 2H, ArH_{2,6}), 7.93 (d, J = 8.8 Hz, 2H, ArH_{3,5}), 8.54 (d, J = 4.8 Hz, 1H, pyrimidine H₆), 10.13 (s, 1H, NH); MS (ESI+) m/z 317 (M+H)⁺; Anal. Calcd for C₁₉H₁₆N₄O: C, 72.13; H, 5.10; N, 17.71. Found: C, 72.20; H, 5.12; N, 17.65.

4.1.5.7. 4-((4-(hydroxy(4-methoxyphenyl)methyl)pyrimidin-2-yl)amino)-benzonitrile (10g). Yield 64.8%; light yellow solid; mp 167.5–168.8 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 3.72 (s, 3H, OCH₃), 5.54 (d, J = 4.4 Hz, 1H, CH), 6.15 (d, J = 4.0 Hz, 1H, OH), 6.90 (d, J = 8.4 Hz, 2H, Ar'H_{3,5}), 7.18 (d, J = 5.2 Hz, 1H, pyrimidine H₅), 7.35 (d, J = 8.8 Hz, 2H, Ar'H_{2,6}), 7.68 (d, J = 8.8 Hz, 2H, Ar'H_{2,6}), 7.93 (d, J = 8.8 Hz, 2H, Ar'H_{3,5}), 8.54 (d, J = 5.2 Hz, 1H, pyrimidine H₆), 10.13 (s, 1H, NH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 55.07, 74.56, 102.21, 109.43, 113.62, 118.23, 119.62, 127.98, 132.92, 135.22, 145.00, 158.64, 158.67, 158.74, 173.87; MS (ESI+) m/z 333 (M+H)⁺; Anal. Calcd for C₁₉H₁₆N₄O₂: C, 68.66; H, 4.85; N, 16.86. Found: C, 68.74; H, 4.87; N, 16.79.

4.1.5.8. 4-((4-((4-tert-butyl)phenyl)(hydroxy)methyl)pyrimidin-2-yl)amino)-benzonitrile (10h). Yield 87.8%; light yellow solid; mp 86.4–88.1 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 1.22 (s, 9H, *t*-Bu), 5.58 (d, J = 4.0 Hz, 1H, CH), 6.19 (d, J = 4.0 Hz, 1H, OH), 7.21 (d, J = 5.2 Hz, 1H, pyrimidine H₅), 7.34–7.40 (m, 4H, Ar'H), 7.68 (d, J = 8.8 Hz, 2H, ArH_{2,6}), 7.96 (d, J = 8.8 Hz, 2H, ArH_{3,5}), 8.55 (d, J = 4.8 Hz, 1H, pyrimidine H₆), 10.15 (s, 1H, NH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 31.11, 34.17, 74.88, 102.27, 109.51, 118.28, 119.64, 124.98, 126.56, 132.90, 140.18, 145.03, 149.84, 158.65, 158.79, 173.75; MS (ESI+) m/z 359 (M+H)⁺; Anal. Calcd for C₂₂H₂₂N₄O: C, 73.72; H, 6.19; N, 15.63. Found: C, 73.81; H, 6.23; N, 15.60.

4.1.5.9. 4-((4-((2-chlorophenyl)(hydroxy)methyl)pyrimidin-2-yl)amino)-benzonitrile (10i). Yield 82.9%; light yellow solid; mp 168.3–170.2 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 5.98 (d, J = 4.8 Hz, 1H, CH), 6.44 (d, J = 4.8 Hz, 1H, OH), 7.19 (d, J = 4.8 Hz, 1H, pyrimidine H₅), 7.34–7.53 (m, 4H, Ar'H), 7.60 (d, J = 8.4 Hz, 2H, ArH_{2,6}), 7.82 (d, J = 8.8 Hz, 2H, ArH_{3,5}), 8.59 (d, J = 4.8 Hz, 1H, pyrimidine H₆), 10.11 (s, 1H, NH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 71.48, 102.22, 110.46, 118.18, 119.57, 127.31, 129.15, 129.21, 132.18, 132.75, 140.55, 144.87, 158.84, 158.96, 171.98; MS (ESI+) m/z 337 (M+H)⁺; Anal. Calcd for C₁₈H₁₃ClN₄O: C, 64.19; H, 3.89; Cl, 10.53; N, 16.64. Found: C, 64.24; H, 3.86; Cl, 10.49; N, 16.63.

4.1.5.10. 4-((4-((2-bromophenyl)(hydroxy)methyl)pyrimidin-2-yl)amino)-benzonitrile (10j). Yield 79.6%; light yellow solid; mp 173.2–175.1 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 5.96 (d, J = 4.8 Hz, 1H, CH), 6.48 (d, J = 4.8 Hz, 1H, OH), 7.19 (d, J = 5.2 Hz, 1H, pyrimidine H₅), 7.24–7.66 (m, 4H, Ar'H), 7.60 (d, J = 8.8 Hz, 2H, ArH_{2,6}), 7.83 (d, J = 8.8 Hz, 2H, ArH_{3,5}), 8.59 (d, J = 5.2 Hz, 1H, pyrimidine H₆), 10.12 (s, 1H, NH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 73.70, 102.19, 110.48, 118.20, 119.56, 122.82, 127.87, 129.46, 129.49, 132.37, 132.75, 142.14, 144.84, 158.82, 159.01, 171.92; MS (ESI+) m/z 381 (M+H)⁺; Anal. Calcd for C₁₈H₁₃BrN₄O: C, 56.71; H, 3.44; Br, 20.96; N, 14.70. Found: C, 56.78; H, 3.46; Br, 20.88; N, 14.65.

4.1.5.11. 4-((4-((2,5-difluorophenyl)(hydroxy)methyl)pyrimidin-2-yl)amino)-benzonitrile (10k). Yield 86.0%; light pink solid; mp 158.5–159.3 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 5.85 (d, J = 4.8 Hz, 1H, CH), 6.53 (d, J = 5.2 Hz, 1H, OH), 7.21–7.30 (m, 4H, pyrimidine H₅ + Ar'H), 7.62 (d, J = 8.8 Hz, 2H, ArH_{2,6}), 7.83 (d, J = 8.8 Hz, 2H, ArH_{3,5}), 8.60 (d, J = 5.2 Hz, 1H, pyrimidine H₆), 10.14 (s, 1H, NH); MS (ESI+) m/z 339 (M+H)⁺; Anal. Calcd for C₁₈H₁₂F₂N₄O: C, 63.90; H, 3.58; F, 11.23; N, 16.56. Found: C, 63.87; H, 3.66; F, 11.14; N, 16.45.

4.1.5.12. 4-((4-((2,5-dimethylphenyl)(hydroxy)methyl)pyrimidin-2-yl)amino)-benzonitrile (10l). Yield 85.2%; light yellow solid; mp 158.7–159.9 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 2.24 (s, 3H, CH₃), 2.33 (s, 3H, CH₃), 5.75 (d, J = 4.0 Hz, 1H, CH), 6.10 (d, J = 4.0 Hz, 1H, OH), 6.97–7.15 (m, 3H, Ar'H), 7.20 (d, J = 5.2 Hz, 1H, pyrimidine H₅), 7.62 (d, J = 8.8 Hz, 2H, ArH_{2,6}), 7.84 (d, J = 8.8 Hz, 2H, ArH_{3,5}), 8.56 (d, J = 5.2 Hz, 1H, pyrimidine H₆), 10.06 (s, 1H, NH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 18.87, 20.70, 71.98, 102.16, 110.02, 118.16, 119.54, 127.74, 127.80, 130.08, 132.30, 132.74, 134.55, 141.22, 144.92, 158.63, 158.68, 173.54; MS (ESI+) m/z 331 (M+H)⁺; Anal. Calcd for C₂₀H₁₈N₄O: C, 72.71; H, 5.49; N, 16.96. Found: C, 72.66; H, 5.57; N, 16.91.

4.1.5.13. 4-((4-((3,4-dichlorophenyl)(hydroxy)methyl)pyrimidin-2-yl)amino)-benzonitrile (10m). Yield 86.0%; light yellow solid; mp 161.7–162.9 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 5.65 (d, J = 4.4 Hz, 1H, CH), 6.51 (d, J = 4.4 Hz, 1H, OH),

7.20 (d, $J = 4.8$ Hz, 1H, pyrimidine H_5), 7.42–7.71 (m, 3H, Ar H), 7.67 (d, $J = 8.8$ Hz, 2H, Ar $H_{2,6}$), 7.90 (d, $J = 8.8$ Hz, 2H, Ar $H_{3,5}$), 8.57 (d, $J = 5.2$ Hz, 1H, pyrimidine H_6), 10.17 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 73.47, 102.38, 109.58, 118.32, 119.58, 127.09, 128.70, 129.99, 130.48, 130.90, 132.87, 144.22, 144.86, 158.79, 159.13, 172.38; MS (ESI+) m/z 371 (M+H) $^+$; Anal. Calcd for $\text{C}_{18}\text{H}_{12}\text{Cl}_2\text{N}_4\text{O}$: C, 58.24; H, 3.26; Cl, 19.10; N, 15.09. Found: C, 58.29; H, 3.36; Cl, 19.01; N, 15.04.

4.1.5.14. 4-((4-((2-fluorophenyl)(hydroxy)methyl)-6-methylpyrimidin-2-yl)amino)benzonitrile (10n). Yield 80.5%; light yellow solid; mp 153.3–154.8 °C; ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 2.43 (s, 3H, CH_3), 5.85 (d, $J = 4.4$ Hz, 1H, CH), 6.33 (d, $J = 4.8$ Hz, 1H, OH), 7.13 (s, 1H, pyrimidine H_5), 7.19–7.48 (m, 4H, Ar H), 7.60 (d, $J = 8.8$ Hz, 2H, Ar $H_{2,6}$), 7.84 (d, $J = 8.8$ Hz, 2H, Ar $H_{3,5}$), 10.13 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 23.86, 68.61 (d, $J_{\text{CF}} = 2.0$ Hz), 102.01, 109.21, 115.22 (d, $J_{\text{CF}} = 22.1$ Hz), 118.06, 119.63, 124.41 (d, $J_{\text{CF}} = 2.9$ Hz), 128.93 (d, $J_{\text{CF}} = 4.6$ Hz), 129.41 (d, $J_{\text{CF}} = 8.0$ Hz), 130.43 (d, $J_{\text{CF}} = 14.0$ Hz), 132.75, 145.11, 158.69, 159.66 (d, $J_{\text{CF}} = 2.9$ Hz), 168.60, 172.11; MS (ESI+) m/z 335 (M+H) $^+$; Anal. Calcd for $\text{C}_{19}\text{H}_{15}\text{FN}_4\text{O}$: C, 68.25; H, 4.52; F, 5.68; N, 16.76. Found: C, 68.33; H, 4.57; F, 5.57; N, 16.69.

4.1.5.15. 4-((4-((2-chlorophenyl)(hydroxy)methyl)-6-methylpyrimidin-2-yl)amino)benzonitrile (10o). Yield 90.9%; light yellow solid; mp 93.2–94.7 °C; ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 2.42 (s, 3H, CH_3), 5.97 (d, $J = 4.4$ Hz, 1H, CH), 6.42 (d, $J = 4.4$ Hz, 1H, OH), 7.10 (s, 1H, pyrimidine H_5), 7.32–7.53 (m, 4H, Ar H), 7.58 (d, $J = 8.8$ Hz, 2H, Ar $H_{2,6}$), 7.82 (d, $J = 8.8$ Hz, 2H, Ar $H_{3,5}$), 10.07 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 23.82, 71.37, 101.98, 109.72, 118.08, 119.63, 127.29, 129.10, 129.18, 132.17, 132.72, 140.73, 145.07, 158.71, 168.63, 171.63; MS (ESI+) m/z 351 (M+H) $^+$; Anal. Calcd for $\text{C}_{19}\text{H}_{15}\text{ClN}_4\text{O}$: C, 65.05; H, 4.31; Cl, 10.11; N, 15.97. Found: C, 64.98; H, 4.38; Cl, 10.09; N, 15.94.

4.1.5.16. 4-((4-((2-bromophenyl)(hydroxy)methyl)-6-methylpyrimidin-2-yl)amino)benzonitrile (10p). Yield 83.8%; light yellow solid; mp 82.8–84.2 °C; ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 2.42 (s, 3H, CH_3), 5.94 (d, $J = 4.8$ Hz, 1H, CH), 6.42 (d, $J = 4.8$ Hz, 1H, OH), 7.10 (s, 1H, pyrimidine H_5), 7.25–7.66 (m, 4H, Ar H), 7.60 (d, $J = 8.8$ Hz, 2H, Ar $H_{2,6}$), 7.84 (d, $J = 8.8$ Hz, 2H, Ar $H_{3,5}$), 10.08 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 23.82, 73.62, 101.96, 109.74, 118.11, 119.64, 122.84, 127.84, 129.42, 129.44, 132.32, 132.72, 142.34, 145.04, 158.69, 168.65, 171.60; MS (ESI+) m/z 397 (M+H) $^+$; Anal. Calcd for $\text{C}_{19}\text{H}_{15}\text{BrN}_4\text{O}$: C, 57.74; H, 3.83; Br, 20.22; N, 14.17. Found: C, 57.67; H, 3.86; Br, 20.17; N, 14.20.

4.1.5.17. 4-((4-((4-fluorophenyl)(hydroxy)methyl)-6-methylpyrimidin-2-yl)amino)benzonitrile (10q). Yield 74.3%; light pink solid; mp 182.3–184.4 °C; ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 2.41 (s, 3H, CH_3), 5.58 (s, 1H, CH), 6.27 (d, $J = 3.6$ Hz, 1H, OH), 7.09 (s, 1H, pyrimidine H_5), 7.16–7.50 (m, 4H, Ar H), 7.67 (d, $J = 8.8$ Hz, 2H, Ar $H_{2,6}$), 7.92 (d, $J = 8.8$ Hz, 2H, Ar $H_{3,5}$), 10.09 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 23.89, 74.16, 102.05, 108.71, 114.95 (d, $J_{\text{CF}} = 21.5$ Hz), 118.16, 119.70, 128.73 (d, $J_{\text{CF}} = 8.1$ Hz), 132.92, 139.55 (d, $J_{\text{CF}} = 2.6$ Hz), 145.15, 158.66, 161.50 (d, $J_{\text{CF}} = 241.2$ Hz), 168.61, 172.95; MS (ESI+) m/z 335 (M+H) $^+$; Anal. Calcd for $\text{C}_{19}\text{H}_{15}\text{FN}_4\text{O}$: C, 68.25; H, 4.52; F, 5.68; N, 16.76. Found: C, 68.36; H, 4.57; F, 5.74; N, 16.68.

4.1.5.18. 4-((4-((hydroxy(phenyl)methyl)-5-methylpyrimidin-2-yl)amino)benzonitrile (10r). Yield 82.6%; light yellow solid; mp 205.4–207.1 °C; ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 2.16 (s, 3H, CH_3), 5.84 (d, $J = 4.4$ Hz, 1H, CH), 6.06 (d, $J = 4.8$ Hz,

1H, OH), 7.28–7.43 (m, 5H, Ar H), 7.63 (d, $J = 8.4$ Hz, 2H, Ar $H_{2,6}$), 7.89 (d, $J = 8.8$ Hz, 2H, Ar $H_{3,5}$), 8.32 (s, 1H, pyrimidine H_6), 10.09 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 14.04, 73.24, 101.70, 117.84, 119.67, 119.94, 126.63, 127.16, 128.08, 132.86, 141.86, 145.20, 157.52, 159.42, 168.88; MS (ESI+) m/z 317 (M+H) $^+$; Anal. Calcd for $\text{C}_{19}\text{H}_{16}\text{N}_4\text{O}$: C, 72.13; H, 5.10; N, 17.71. Found: C, 72.08; H, 5.17; N, 17.67.

4.1.5.19. 4-((4-((2-chlorophenyl)(hydroxy)methyl)-5-methylpyrimidin-2-yl)amino)benzonitrile (10s). Yield 88.4%; light yellow solid; mp 202.6–204.3 °C; ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 2.36 (s, 3H, CH_3), 6.10 (d, $J = 6.0$ Hz, 1H, CH), 6.33 (d, $J = 6.0$ Hz, 1H, OH), 7.41–7.85 (m, 8H, Ar H + Ar H), 8.41 (s, 1H, pyrimidine H_6); ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 13.88, 69.36, 101.56, 117.63, 119.68, 120.72, 126.94, 128.73, 128.85, 129.14, 131.14, 132.60, 140.34, 145.09, 157.56, 159.61, 167.15; MS (ESI+) m/z 351 (M+H) $^+$; Anal. Calcd for $\text{C}_{19}\text{H}_{15}\text{ClN}_4\text{O}$: C, 65.05; H, 4.31; Cl, 10.11; N, 15.97. Found: C, 65.02; H, 4.39; Cl, 10.07; N, 15.94.

4.1.5.20. 4-((4-((4-chlorophenyl)(hydroxy)methyl)-5-methylpyrimidin-2-yl)amino)benzonitrile (10t). Yield 84.8%; light yellow solid; mp 187.7–188.6 °C; ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 2.19 (s, 3H, CH_3), 5.86 (s, 1H, CH), 6.19 (d, $J = 2$ Hz, 1H, OH, deuterium exchange), 7.44 (s, 4H, Ar H), 7.61 (d, $J = 8.8$ Hz, 2H, Ar $H_{2,6}$), 7.83 (d, $J = 8.4$ Hz, 2H, Ar $H_{3,5}$), 8.34 (s, 1H, pyrimidine H_6), 10.08 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 14.02, 73.50, 101.74, 117.84, 119.67, 120.13, 128.05, 128.54, 131.70, 132.80, 141.10, 145.17, 157.52, 159.61, 168.41; MS (ESI+) m/z 351 (M+H) $^+$; Anal. Calcd for $\text{C}_{19}\text{H}_{15}\text{ClN}_4\text{O}$: C, 65.05; H, 4.31; Cl, 10.11; N, 15.97. Found: C, 64.98; H, 4.39; Cl, 10.07; N, 15.92.

4.1.5.21. 4-((4-((3-fluorophenyl)(hydroxy)methyl)-5-methylpyrimidin-2-yl)amino)benzonitrile (10u). Yield 83.9%; light yellow solid; mp 169.4–170.8 °C; ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 2.20 (s, 3H, CH_3), 5.88 (d, $J = 5.2$ Hz, 1H, CH), 6.26 (d, $J = 5.2$ Hz, 1H, OH), 7.11–7.43 (m, 4H, Ar H), 7.61 (d, $J = 8.8$ Hz, 2H, Ar $H_{2,6}$), 7.84 (d, $J = 8.4$ Hz, 2H, Ar $H_{3,5}$), 8.35 (s, 1H, pyrimidine H_6), 10.10 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 14.06, 72.55, 101.77, 113.37 (d, $J_{\text{CF}} = 21.5$ Hz), 113.92 (d, $J_{\text{CF}} = 20.8$ Hz), 117.84, 119.71, 120.19, 122.70 (d, $J_{\text{CF}} = 2.9$ Hz), 130.07 (d, $J_{\text{CF}} = 8.1$ Hz), 132.84, 145.16 (d, $J_{\text{CF}} = 7.3$ Hz), 157.58, 159.68, 162.20 (d, $J_{\text{CF}} = 241.9$ Hz), 168.32; MS (ESI+) m/z 335 (M+H) $^+$; Anal. Calcd for $\text{C}_{19}\text{H}_{15}\text{FN}_4\text{O}$: C, 68.25; H, 4.52; F, 5.68; N, 16.76. Found: C, 68.21; H, 4.60; F, 5.63; N, 16.72.

4.1.5.22. 4-((4-((4-fluorophenyl)(hydroxy)methyl)-5-methylpyrimidin-2-yl)amino)benzonitrile (10v). Yield 79.5%; light yellow solid; mp 181.1–181.8 °C; ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 2.17 (s, 3H, pyrimidine- CH_3), 5.86 (s, 1H, CH), 6.15 (s, 1H, OH), 7.17–7.47 (m, 4H, Ar H), 7.63 (d, $J = 8.8$ Hz, 2H, Ar $H_{2,6}$), 7.88 (d, $J = 8.4$ Hz, 2H, Ar $H_{3,5}$), 8.32 (s, 1H, pyrimidine H_6), 10.12 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 14.08, 72.51, 101.78, 114.89 ($J_{\text{CF}} = 21.0$ Hz), 117.86, 119.75, 119.99, 128.70 ($J_{\text{CF}} = 8.1$ Hz), 132.90, 138.16 ($J_{\text{CF}} = 3.1$ Hz), 145.24, 157.61, 159.57, 161.44 ($J_{\text{CF}} = 241.3$ Hz), 168.67; MS (ESI+) m/z 335 (M+H) $^+$; Anal. Calcd for $\text{C}_{19}\text{H}_{15}\text{FN}_4\text{O}$: C, 68.25; H, 4.52; F, 5.68; N, 16.76. Found: C, 68.19; H, 4.63; F, 5.61; N, 16.74.

4.1.5.23. 4-((4-((2-bromophenyl)(hydroxy)methyl)-5-methylpyrimidin-2-yl)amino)benzonitrile (10w). Yield 80.3%; light yellow solid; mp 174.1–175.5 °C; ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 2.21 (s, 3H, CH_3), 5.85 (s, 1H, CH), 6.22 (s, 1H, OH), 7.33–7.64 (m, 6H, Ar $H_{2,6}$ + Ar H), 7.82 (d, $J = 8.8$ Hz, 2H, Ar $H_{3,5}$), 8.34 (s, 1H, pyrimidine H_6), 10.08 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 14.01, 72.55, 101.73, 117.82, 119.65, 120.15, 120.22, 128.89, 130.94, 132.78, 141.54, 145.15, 157.49, 159.59,

168.34; MS (ESI+) m/z 397 ($M^+ + 1$), Calcd for $C_{19}H_{15}BrN_4O$: C, 57.74; H, 3.83, Br 20.22; N, 14.17. Found: C, 57.67; H, 3.86, Br 20.20; N, 14.13.

4.1.5.24. 4-((4-(hydroxy(*p*-tolyl)methyl)-5-methylpyrimidin-2-yl)amino)benzonitrile (10x). Yield 79.5%; light yellow solid; mp 182.7–184.3 °C; 1H NMR (400 MHz, DMSO- d_6) δ (ppm): 2.13 (s, 3H, Ar'-CH₃), 2.28 (s, 3H, pyrimidine-CH₃), 5.80 (s, 1H, CH), 5.99 (s, 1H, OH), 7.15 (d, J = 8.0 Hz, 2H, Ar' $H_{3,5}$), 7.29 (d, J = 8.0 Hz, 2H, Ar' $H_{2,6}$), 7.64 (d, J = 8.8 Hz, 2H, Ar' $H_{2,6}$), 7.93 (d, J = 8.8 Hz, 2H, Ar' $H_{3,5}$), 8.30 (s, 1H, pyrimidine H_6), 10.10 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 14.03, 20.66, 73.12, 101.73, 117.87, 119.68, 119.79, 126.57, 128.65, 132.86, 136.26, 138.81, 145.23, 157.52, 159.30, 169.02; MS (ESI+) m/z 331 ($M+H$)⁺; Anal. Calcd for $C_{20}H_{18}N_4O$: C, 72.71; H, 5.49; N, 16.96. Found: C, 72.59; H, 5.56; N, 16.93.

4.1.5.25. 4-((4-(hydroxy(*m*-tolyl)methyl)-5-methylpyrimidin-2-yl)amino)benzonitrile (10y). Yield 84.1%; light yellow solid; mp 198.6–199.8 °C; 1H NMR (400 MHz, DMSO- d_6) δ (ppm): 2.15 (s, 3H, Ar'-CH₃), 2.28 (s, 3H, pyrimidine-CH₃), 5.79 (d, J = 5.2 Hz, 1H, CH), 6.00 (d, J = 4.8 Hz, 1H, OH), 7.08–7.26 (m, 4H, Ar' H), 7.64 (d, J = 8.8 Hz, 2H, Ar' $H_{2,6}$), 7.91 (d, J = 8.8 Hz, 2H, Ar' $H_{3,5}$), 8.31 (s, 1H, pyrimidine H_6), 10.09 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 14.07, 21.09, 73.24, 101.72, 117.85, 119.67, 119.89, 123.76, 127.13, 127.78, 127.98, 132.85, 137.12, 141.78, 145.22, 157.51, 159.36, 168.91; MS (ESI+) m/z 331 ($M+H$)⁺; Anal. Calcd for $C_{20}H_{18}N_4O$: C, 72.71; H, 5.49; N, 16.96. Found: C, 72.67; H, 5.54; N, 16.90.

4.1.5.26. 4-((4-(hydroxy(*o*-tolyl)methyl)-5-methylpyrimidin-2-yl)amino)benzonitrile (10z). Yield 85.7%; light yellow solid; mp 222.8–224.3 °C; 1H NMR (400 MHz, DMSO- d_6) δ (ppm): 2.13 (s, 3H, Ar'-CH₃), 2.23 (s, 3H, pyrimidine-CH₃), 5.97 (s, 2H, OH + CH, 1H was deuterium-exchanged), 7.14–7.51 (m, 6H, Ar' $H_{2,6}$ + Ar' H), 7.67 (d, J = 8.8 Hz, 2H, Ar' $H_{3,5}$), 8.36 (s, 1H, pyrimidine H_6), 10.02 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 14.98, 18.89, 70.00, 101.56, 117.78, 119.73, 120.48, 125.56, 126.98, 127.05, 129.90, 132.71, 134.95, 140.44, 145.20, 157.55, 159.40, 168.13; MS (ESI+) m/z 331 ($M+H$)⁺; Anal. Calcd for $C_{20}H_{18}N_4O$: C, 72.71; H, 5.49; N, 16.96. Found: C, 72.69; H, 5.56; N, 16.92.

4.2. Anti-HIV activity assay

The anti-HIV activity and cytotoxicity of the compounds were evaluated against wild-type HIV-1 strain III_B, double RT mutant (K103N + Y181C) HIV-1 and HIV-2 strain ROD in MT-4 cell cultures using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.¹⁵ 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 viral infection, 100 μ L of the cell suspension was placed in each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. The tested compounds were dissolved in DMSO at 50 mM. After 4 days of incubation 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.

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