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Synthesis and biological evaluation of 4-(hydroxyimino)arylmethyl diarylpyrimidine analogues as potential non-nucleoside reverse transcriptase inhibitors against HIV

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

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

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ABSTRACT

A series of novel diarylpyrimidine analogues featuring a hydroxyiminomethyl group between the pyrimidine scaffold and the aryl wing I have been synthesized and tested in MT-4 cells culture as non-nucleoside reverse transcriptase inhibitors against human immunodeficiency virus (HIV). Most of these new congeners exhibited moderate to excellent activity against wild-type virus with an EC_{50} value ranging from 0.569 μM to 0.005 μM. 4-(4-((Hydroxyimino) (3-methoxyphenyl)methyl)pyrimidin-2-ylamino)benzonitrile (**12n**) was identified as the most active compound of this new series (EC_{50} = 0.025 μM, SI >1223) associated with moderate activity against HIV-1 double mutant strains (K103N + Y181C) (EC_{50} = 8.72 μM) in addition to its anti-HIV-2 activity with an EC_{50} value of 8.31 μM. Preliminary structure–activity relationship (SAR) among the newly synthesized DAPYs was also investigated.

1. Introduction

Progress in the last decade has been achieved by marked advances in the chemical modifications of pyrimidine scaffold, aryl wing I and the linker between the pyrimidine nucleus and the aryl moiety at C-4 position of the diarylpyrimidine analogues (DAPYs) (Fig. 1).[1–5] A panel of novel DAPY derivatives including *S*-DAPYs, *NH*-DAPYs, and *O*-DAPYs were identified as non-nucleoside reverse transcriptase inhibitors (NNRTIs) with strong potency against wild-type and clinically mutant strains of human immunodeficiency virus (HIV) (**1**–**6**, Fig. 1).[1–5] In the course of our studies on the development of new NNRTIs, we have recently synthesized a series of 4-(4-(substituted-naphthalen-1or 2-yloxy)pyrimidin-2-ylamino)benzonitriles in which the phenyl wing I was replaced by a naphthalene ring (**7**, Fig. 1).[6,7] This series of compounds exhibited high activity and very low cytotoxicity against HIV-1. As part of our ongoing efforts to identify potent and selective DAPY inhibitors, we decided to examine whether the introduction of a hydroxyimino group onto the CH_2 linker between the pyrimidine nucleus and the aryl wing I of the DAPYs could reflect on an improved biological activity against HIV-1 and offer a structurally novel scaffold for the future development of HIV-1 inhibitors (**8**, Fig. 1). In this paper, we describe the synthesis, biological evaluation of anti-HIV activity and preliminary structure–activity relationship (SAR) of these new DAPY congeners.

2. Chemistry

The synthesis of the newly designed DAPYs **12a–s** is depicted in Scheme 1. The known 4-(4-chloropyrimidin-2-ylamino)benzonitriles (**9**) were conveniently prepared by refluxing of 4-(4-hydroxypyrimidin-2-ylamino)benzonitriles with $POCl_3$ according to our reported procedure.[6] Treatment of **9** with the corresponding arylacetonitriles in the presence of 60% NaH in anhydrous DMF at room temperature for 24–72 h under N_2 atmosphere afforded 4-(4-(cyanoarylmethyl)pyrimidin-2-ylamino)benzonitriles (**10a–s**), which were subjected to oxidation by bubbling air at room temperature to yield the 4-(4-aroylpyrimidin-2-ylamino)benzonitriles (**11a–s**).[8] Oxidation of **11a–s** with hydroxylamine hydrochloride in the presence of NaOH in EtOH and H_2O provided the title 4-(4-((hydroxyimino)arylmethyl)pyrimidin-2-ylamino)benzonitriles (**12a–s**) in 20–60% yield.[9]

3. Results and discussion

All the 4-(4-((hydroxyimino)arylmethyl)pyrimidin-2-ylamino)benzonitriles **12a–s** were assayed according to the MTT method in MT-4 cells for their biological activity against wild-type HIV-1 strain III_B and HIV-2 strain ROD along with the double mutant

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

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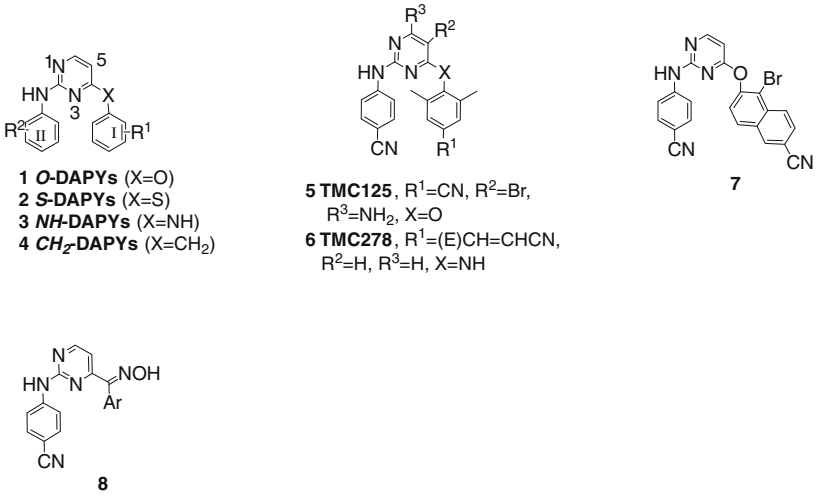
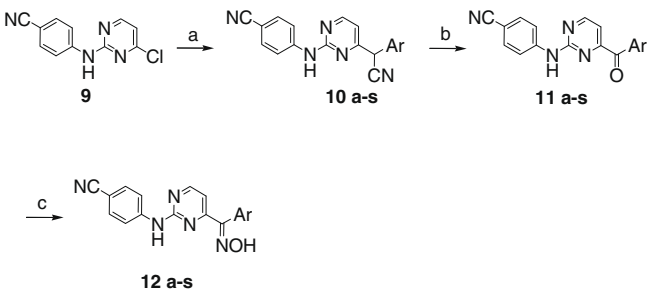


Figure 1. Chemical structures of DAPYs.

strains RES056 (K103N + Y181C). The biological evaluation is summarized in Table 1. Efavirenz (EFV), delavirdine (DEV), and nevirapine (NEV), three drugs currently used in clinical treatment of HIV-1 infection, were also tested as reference compounds.

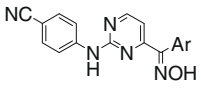
Table 1 shows the results of anti-HIV activities of the newly synthesized DAPY analogues containing hydroxyimino group between the pyrimidine scaffold and the aryl wing I. All the newly synthesized compounds inhibited the replication of HIV-1 effectively at lower micromolar concentration range from 0.827 μM to 0.005 μM. Except for the compound **12d**, all of the substituted-phenyl DAPYs conferred more potent activity than the non-substituted parent compound **12a** (EC_{50} = 0.569 μM). It is worth noting that striking variable SI values of these new DAPYs are observed from 24.57 to 3791. In general, compound **12n** was identified as the most active compound of this series (EC_{50} = 0.025 μM, SI >1223), along with moderate activity against double mutant strains RES056 (K103N + Y181C) with an EC_{50} value of 8.72 μM. Differing from other typical NNRTIs, **12n** still displayed moderate activity against HIV-2 ROD strains (EC_{50} = 8.31 μM). In comparison with the reference drugs tested, compound **12n** was 8-fold and 29-fold more potent than NEV and DEV against HIV-1 III_B strains, respectively.

To explore the potential SAR among the newly designed analogues, this series of DAPYs with various substituents on wing I were synthesized and assayed for their in vitro anti-HIV bioactivity. As seen from Table 1, the introduction of a halogen atom onto the *ortho* position of the phenyl ring resulted in compounds **12b**, **12e**, and **12h** with relatively excellent activity of 0.053 μM, 0.013 μM, and 0.005 μM, respectively. In contrast, compound **12k** (EC_{50} = 0.048 μM) with a methyl group at the *ortho* position displayed slightly less potency than the compounds with corresponding substituents at the *meta* or *para* position **12l** (EC_{50} = 0.032 μM) and **12m** (EC_{50} = 0.006 μM). For the *meta* or *para* substitutions, in general, *para* substituted compounds were more active than the *meta* substituted ones except for the fact that varying the fluorine atom from *meta* to *para* position dramatically decreased the activity from 0.225 μM to 0.827 μM. In light of the above findings that introducing a methyl group at the *para* position of the phenyl ring



Scheme 1. Reagents and conditions: (a) Ar-CH_2CN, 60% NaH, DMF, rt, 24–72 h, N_2; (b) air, DMF, rt, 48–72 h; (c) $H_2NOH{\cdot}HCl$, NaOH, EtOH, H_2O, reflux, 1 h.

Table 1
Biological activities of compounds **12a**–**s** against HIV-1 in MT-4 cells[a]



	Ar	EC_{50}[b] (μM)		CC_{50}[d] (μM)	SI[e]
		III_B	RES056[c]		
12a	Ph	0.569 ± 0.404	5.60	13.99 ± 9.51	24.57
12b	2-F-Ph	0.053 ± 0.007	>5.67	5.67 ± 0.66	106.37
12c	3-F-Ph	0.225 ± 0.011	⩾6.70	15.30 ± 18.17	67.87
12d	4-F-Ph	0.827 ± 0.124	13.85	>377.26	>456
12e	2-Cl-Ph	0.013 ± 0.003	>12.62	12.62 ± 8.85	997.16
12f	3-Cl-Ph	0.298 ± 0.095	>14.92	30.44 ± 1.63	102.10
12g	4-Cl-Ph	0.083 ± 0.007	⩾7.62	17.24 ± 13.50	208.52
12h	2-Br-Ph	0.005 ± 0.003	>13.23	13.23 ± 10.60	2501.20
12i	3-Br-Ph	0.362 ± 0.198	>23.53	23.54 ± 2.97	64.97
12j	4-Br-Ph	0.127 ± 0.058	⩾7.21	26.01 ± 5.62	204.87
12k	2-Me-Ph	0.048 ± 0.001	⩾1.59	29.89 ± 3.98	619.30
12l	3-Me-Ph	0.032 ± 0.019	4.52	19.96 ± 12.76	626.26
12m	4-Me-Ph	0.006 ± 0.005	⩾5.10	13.27 ± 7.31	2394.60
12n	3-OMe-Ph	0.025 ± 0.006	8.72	30.51 ± 1.43	1223.00
12o	4-OMe-Ph	0.013 ± 0.005	10.29	50.24 ± 15.94	3791.21
12p	4-*t*-Butyl-Ph	0.144 ± 0.074	7.20	33.66 ± 7.35	233.57
12q	3,5-DiMe-Ph	0.047 ± 0.005	7.89	35.88 ± 2.91	756.17
12r	1-Naphthyl	0.039 ± 0.009	>33.10	33.09 ± 1.41	843.86
12s	2-Naphthyl	0.047 ± 0.011	>14.28	31.64 ± 2.26	672.51
EFV		0.003 ± 0.0006	0.18 ± 0.08	>6.34	>1843
DEV		0.72 ± 0.07	>43.81	>43.81	>62
NEV		0.20 ± 0.11	>15.02	>15.02	>75

[a] All data represent mean values for at least two separate experiments.
[b] EC_{50}: effective concentration of compound required to protect the cell against viral cytopathogenicity by 50% in MT-4 cells.
[c] RES056: HIV-1 mutated strain bearing both K103N and Y181C mutations.
[d] CC_{50}: cytotoxic concentration of compound that reduces the uninfected MT-4 cell viability by 50%.
[e] SI: selectivity index: ratio CC_{50}/EC_{50}.

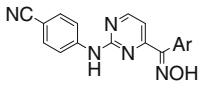
led to excellent activity with an EC_{50} value of 0.006 μM, compounds **12o**, **12p** and **12q** with *para*-OCH_3, *para*-*t*-butyl and 3,5-diMe group on the phenyl ring were synthesized and evaluated. Compounds **12o** (EC_{50} = 0.013 μM), **12p** (EC_{50} = 0.144 μM) and **12q** (EC_{50} = 0.047 μM) afforded a slight loss of potency compared with the parent compound **12m** (EC_{50} = 0.006 μM), indicating that methyl might have the appropriate bulk to accommodate the hydrophobic pocket formed by Y181, Y188 and W229 of the RT.

In our previous research, based on the hypothesis that replacing the phenyl wing I of DAPYs with a naphthyl group might enhance $\pi\sim$ stacking interaction between the inhibitors and the amino acid residues Tyr181, Tyr188, and Trp229 within the binding pocket of RT,[10] a panel of compounds endowed with excellent potency against HIV were achieved.[6–8,11,12] Then, promoted by the intriguing observations, compounds **12r** and **12s** were successfully synthesized and assayed. The results indicated that they possessed comparable activity (EC_{50} = 0.039 μM and EC_{50} = 0.047 μM, respectively) with the substituted-phenyl DAPYs implying that they might lead to another series of DAPYs with potent anti-HIV activity.

In addition, we further evaluated the capability of the compounds to inhibit the multiplication of HIV-1 strains (RES056) bearing both K103N and Y181C mutations in cell culture (Table 1). It was found that *meta* or *para* substituted-phenyl DAPYs displayed moderate activity against double mutant strains in the low micromolar range, while the *ortho* position substituted ones almost lost their activity entirely. Among the tested compounds, **12n** was the most potent inhibitor exhibiting activity against the mutant strains at low micromolar concentration with an EC_{50} value of 8.72 μM. Although less active than EFV, compound **12n** was still superior to DEV and NEV which disclosed a promising perspective for this series of derivatives.

Furthermore, all of the newly synthesized compounds **12a–s** were tested for their bioactivity against HIV-2 (strain ROD) and the results are listed in Table 2. Unfortunately, none of them exhibited activity except for **12d** and **12n** displaying moderate potency at micromolar concentration (EC_{50} = 19.90 μM, EC_{50} = 8.31 μM, respectively).

Table 2
Biological activities of compounds **12a–s** against HIV-2 in MT-4 cells[a]



	Ar	EC_{50}[b] (μM)	CC_{50}[c] (μM)	SI[d]
12a	Ph	⩾13.98	13.99 ± 9.51	<1
12b	2-F-Ph	>5.67	5.67 ± 0.66	<1
12c	3-F-Ph	⩾8.33	15.30 ± 18.17	⩽2
12d	4-F-Ph	19.90	>377.26	>19
12e	2-Cl-Ph	>12.62	12.62 ± 8.85	<1
12f	3-Cl-Ph	⩾7.30	30.44 ± 1.63	⩽4
12g	4-Cl-Ph	⩾7.33	17.24 ± 13.50	⩽2
12h	2-Br-Ph	>13.23	13.23 ± 10.60	<1
12i	3-Br-Ph	>23.53	23.54 ± 2.97	<1
12j	4-Br-Ph	⩾4.97	26.01 ± 5.62	⩽5
12k	2-Me-Ph	>29.90	29.89 ± 3.98	<1
12l	3- Me-Ph	⩾11.55	19.96 ± 12.76	<1
12m	4-Me-Ph	⩾6.54	13.27 ± 7.31	⩽2
12n	3-OMe-Ph	8.31	30.51 ± 1.43	3.67
12o	4-OMe-Ph	⩾4.72	50.24 ± 15.94	⩽11
12p	4-*t*-Butyl-Ph-Ph	⩾33.67	33.66 ± 7.35	<1
12q	3,5-DiMe-Ph	⩾8.96	35.88 ± 2.91	⩽4
12r	1-Naphthyl	>33.10	33.09 ± 1.41	<1
12s	2-Naphthyl	>14.28	31.64 ± 2.26	<1

[a] All data represent mean values for at least two separate experiments.
[b] EC_{50}: effective concentration of compound required to protect the cell against viral cytopathogenicity by 50% in MT-4 cells.
[c] CC_{50}: cytotoxic concentration of compound that reduces the normal uninfected MT-4 cell viability by 50%.
[d] SI: selectivity index: ratio CC_{50}/EC_{50}.

4. Conclusions

In this study, a series of novel diarylpyrimidine analogues (DAPYs) containing a hydroxyiminomethyl group between the pyrimidine scaffold and the aryl wing I have been synthesized and evaluated for their in vitro bioactivity against human immunodeficiency virus. Their preliminary structure–activity relationship was also investigated. Among all the compounds (**12a–s**) examined, **12n** was identified as the most active compound (EC_{50} = 0.025 μM, SI >1223) associated with moderate activity against HIV-1 double mutant strains (K103N + Y181C) (EC_{50} = 8.72 μM) in addition to its anti-HIV-2 activity with an EC_{50} value of 8.31 μM. The bioactivity test results indicated that the linker between the pyrimidine scaffold and the aryl wing I played an essential role in the interaction between the inhibitors and RT so that it should deserve further modification which might lead to even more potent compounds against HIV.

5. Experimental

5.1. Chemistry

5.1.1. General

Chemical reagents and solvents, purchased from commercial sources, were of analytical grade and were used without further purification. Melting points were determined on a WRS-1 digital melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Brucker AV 400 MHz spectrometer were recorded in DMSO-d_6, and the chemical shifts are reported in δ (ppm) units relative to the internal standard tetramethylsilane (TMS). EI Mass spectra were obtained on an Agilent MS/5975 spectrometer. All the progress of the reactions was monitored by thin-layer chromatography (TLC) on pre-coated silica gel G plates at 254 nm under a UV lamp using ethyl acetate/hexane as eluents. Flash chromatography separations were performed on silica gel (300–400 mesh).

5.1.2. General procedure for the preparation of 4-(4-aroylpyrimidin-2-ylamino)benzonitriles (11a–s)

To a solution of **9** (1.4 g, 6 mmol) in anhydrous DMF (30 mL) was added appropriate aryl acetonitriles (12 mmol). After stirred at −10 °C for about 15 min, 60% NaH (0.48 g, 12 mmol) was added portionwise under a nitrogen atmosphere and maintained at this condition for 1 h. Then, the whole solution was warmed slowly to room temperature and continued to react for 24–72 h. Air was passed through the solution for 48–72 h. The resulting mixture was poured into 300 ml H_2O and neutralized with 3 N HCl. The precipitate was collected for the next step without further purification.

5.1.3. General procedure for the preparation of 4-(4-((hydroxyimino) arylmethyl) pyrimidin-2-ylamino)benzonitriles (12a–s)

To a violently stirred solution of **11a–s** (6 mmol) in EtOH (50 mL) and H_2O (20 mL) was added NaOH (4.0 g, 100 mmol) and hydroxylamine hydrochloride (2.1 g, 30 mmol). After refluxed for 1.5 h, the resulting solution was poured into 300 mL H_2O and neutralized with 3 N HCl. The filtrated precipitation was dried. The product was purified by flash chromatography to give the pure target compounds **12a–s**.

5.1.4. 4-(4-((Hydroxyimino)(phenyl)methyl)pyrimidin-2-ylamino)benzonitrile (12a)

Yield 23.6%; light yellow solid, mp 208.8–212.3 °C; ^{1}H NMR (DMSO-d_6) δ: 7.42 (d, 1H, *J* = 5.2 Hz, C*H*), 7.37–7.67 (m, 9H, Ph*H*),

8.61 (d, 1H, J = 5.2 Hz, C*H*), 10.16 (s, 1H, N*H*), 12.17 (s, 1H, NO*H*); ^{13}C NMR (DMSO-d_6) δ: 102.6, 110.1, 118.6 (2C), 120.0, 128.4 (2C), 128.9 (2C), 129.7, 132.5, 133.0 (2C), 145.2, 155.1, 159.1, 159.5, 163.2; MS (EI) m/z 315.1 (M^+), calcd for $C_{18}H_{13}N_5O$ 315.3.

5.1.5. 4-(4-((2-Fluorophenyl)(hydroxyimino)methyl)pyrimidin-2-ylamino) benzonitrile (12b)

Yield 28.7%; yellow solid, mp 205.6–206.1 °C; ^{1}H NMR (DMSO-d_6) δ: 7.47 (d, 1H, J = 5.2 Hz, C*H*), 7.35–7.66 (m, 8H, Ph*H*), 8.62 (d, 1H, J = 5.2 Hz, C*H*), 10.18 (s, 1H, N*H*), 12.50 (s, 1H, NO*H*); ^{13}C NMR (DMSO-d_6) δ: 102.7, 109.1, 116.0, 118.5 (2C), 119.9, 124.7(2C), 131.3, 131.4, 133.0 (2C), 145.1, 151.0, 159.3, 159.6, 160.6, 162.1; MS (EI) m/z 333.1 (M^+), calcd for $C_{18}H_{12}FN_5O$ 333.3.

5.1.6. 4-(4-((3-Fluorophenyl)(hydroxyimino)methyl)pyrimidin-2-ylamino)benzonitrile (12c)

Yield 19.8%; light yellow solid, mp 195.9–196.8 °C; ^{1}H NMR (DMSO-d_6) δ: 7.43 (d, 1H, J = 5.2 Hz, C*H*), 7.20–7.68 (m, 8H, Ph*H*), 8.62 (d, 1H, J = 5.2 Hz, C*H*), 10.18 (s, 1H, N*H*), 12.35 (s, 1H, NO*H*); ^{13}C NMR (DMSO-d_6) δ: 102.7, 110.0, 115.7, 116.8, 118.6 (2C), 120.0, 126.0, 130.4, 133.0 (2C), 134.6, 145.1, 154.0, 159.1, 159.5, 162.7, 163.5; MS (EI) m/z 333.1 (M^+), calcd for $C_{18}H_{12}FN_5O$ 333.3.

5.1.7. 4-(4-((4-Fluorophenyl)(hydroxyimino)methyl)pyrimidin-2-ylamino) benzonitrile (12d)

Yield 24.6%; light yellow solid, mp 220.7–222.5 °C; ^{1}H NMR (DMSO-d_6) δ: 7.41 (d, 1H, J = 5.2 Hz, C*H*), 7.34–7.72 (m, 8H, Ph*H*), 8.62 (d, 1H, J = 5.2 Hz, C*H*), 10.16 (s, 1H, N*H*), 12.26 (s, 1H, NO*H*); ^{13}C NMR (DMSO-d_6) δ: 102.7, 110.2, 115.4 (2C), 118.7 (2C), 120.0, 128.5, 132.3 (2C), 133.0 (2C), 145.2, 154.1, 159.1, 159.5, 161.3, 163.7; MS (EI) m/z 333.1 (M^+), calcd for $C_{18}H_{12}FN_5O$ 333.3.

5.1.8. 4-(4-((2-Chlorophenyl)(hydroxyimino)methyl)pyrimidin-2-ylamino) benzonitrile (12e)

Yield 20.0%; light yellow solid, mp 186.9–188.0 °C; ^{1}H NMR (DMSO-d_6) δ: 7.44 (d, 1H, J = 5.2 Hz, C*H*), 7.32–7.63 (m, 8H, Ph*H*), 8.58 (d, 1H, J = 5.2 Hz, C*H*), 10.15 (s, 1H, N*H*), 12.36 (s, 1H, NO*H*); ^{13}C NMR (DMSO-d_6) δ: 102.6, 108.9, 118.4 (2C), 120.0, 127.5, 129.5, 130.7, 131.1, 132.1, 133.0 (2C), 139.8, 145.2, 153.6, 159.3, 159.6, 161.9; MS (EI) m/z 349.1 (M^+), calcd for $C_{18}H_{12}ClN_5O$ 349.8.

5.1.9. 4-(4-((3-Chlorophenyl)(hydroxyimino)methyl)pyrimidin-2-ylamino) benzonitrile (12f)

Yield 28.9%; light yellow solid, mp 189.5–191.0 °C; ^{1}H NMR (DMSO-d_6) δ: 7.45 (d, 1H, J = 5.2 Hz, C*H*), 7.08–8.00 (m, 8H, Ph*H*), 8.64 (d, 1H, J = 5.2 Hz, C*H*), 10.42 (s, 1H, N*H*), 12.38 (s, 1H, NO*H*); ^{13}C NMR (DMSO-d_6) δ: 103.4, 111.9, 118.6 (2C), 119.9, 122.7, 129.1, 131.3, 132.1, 133.4 (2C), 135.7, 137.1, 144.9, 148.4, 159.8, 160.6, 164.4; MS (EI) m/z 349.1 (M^+), calcd for $C_{18}H_{12}ClN_5O$ 349.8.

5.1.10. 4-(4-((4-Chlorophenyl)(hydroxyimino)methyl) pyrimidin-2-ylamino) benzonitrile (12g)

Yield 30.5%; yellow solid, mp 221.9–223.1 °C; ^{1}H NMR (DMSO-d_6) δ: 7.42 (d, 1H, J = 5.2 Hz, C*H*), 7.41–7.69 (m, 8H, Ph*H*), 8.61 (d, 1H, J = 5.2 Hz, C*H*), 10.16 (s, 1H, N*H*), 12.33 (s, 1H, NO*H*); ^{13}C NMR (DMSO-d_6) δ: 102.7, 110.0, 118.7 (2C), 120.0, 128.5 (2C), 131.3 (2C), 131.8, 133.0 (2C), 133.6, 145.2, 154.1, 159.1, 159.4, 162.8; MS (EI) m/z 349.1 (M^+), calcd for $C_{18}H_{12}ClN_5O$ 349.8.

5.1.11. 4-(4-((2-Bromophenyl)(hydroxyimino)methyl) pyrimidin-2-ylamino) benzonitrile (12h)

Yield 26.9%; light yellow solid, mp 189.3–190.8 °C; ^{1}H NMR (DMSO-d_6) δ: 7.44 (d, 1H, J = 5.2 Hz, C*H*), 7.29–7.79 (m, 8H, Ph*H*), 8.58 (d, 1H, J = 5.2 Hz, C*H*), 10.15 (s, 1H, N*H*), 12.33 (s, 1H, NO*H*); ^{13}C NMR (DMSO-d_6) δ: 102.6, 109.0, 118.4 (2C), 120.0, 127.5, 129.5, 130.7, 131.1, 132.1, 133.0 (2C), 135.2, 145.2, 154.7, 159.3, 159.5, 161.8; MS (EI) m/z 393.0 (M^+), calcd for $C_{18}H_{12}BrN_5O$ 394.2.

5.1.12. 4-(4-((3-Bromophenyl)(hydroxyimino)methyl) pyrimidin-2-ylamino) benzonitrile (12i)

Yield 36.5%; light yellow solid, mp 220.2–222.1 °C; ^{1}H NMR (DMSO-d_6) δ: 7.44 (d, 1H, J = 5.2 Hz, C*H*), 7.08–7.99 (m, 8H, Ph*H*), 8.64 (d, 1H, J = 5.2 Hz, C*H*), 10.42 (s, 1H, N*H*), 12.38 (s, 1H, NO*H*); ^{13}C NMR (DMSO-d_6) δ: 103.4, 111.9, 118.9 (2C), 120.0, 125.8, 129.1, 132.0, 132.1, 133.4 (2C), 133.7, 137.1, 144.9, 157.6, 159.8, 160.6, 164.4; MS (EI) m/z 393.1 (M^+), calcd for $C_{18}H_{12}BrN_5O$ 394.2.

5.1.13. 4-(4-((4-Bromophenyl)(hydroxyimino)methyl) pyrimidin-2-ylamino) benzonitrile (12j)

Yield 21.3%; light yellow solid, mp 208.7–210.2 °C; ^{1}H NMR (DMSO-d_6) δ: 7.44 (d, 1H, J = 5.2 Hz, C*H*), 7.35–7.76 (m, 8H, Ph*H*), 8.62 (d, 1H, J = 5.2 Hz, C*H*), 10.17 (s, 1H, N*H*), 12.34 (s, 1H, NO*H*); ^{13}C NMR (DMSO-d_6) δ: 102.7, 109.9, 118.7 (2C), 120.0, 125.4, 131.4 (2C), 131.7 (2C), 132.0, 133.0 (2C), 145.1, 154.2, 159.1, 159.4, 162.7; MS (EI) m/z 393.1 (M^+), calcd for $C_{18}H_{12}BrN_5O$ 394.2.

5.1.14. 4-(4-((Hydroxyimino)(*o*-tolyl)methyl)pyrimidin-2-ylamino)benzonitrile (12k)

Yield 30.7%; light yellow solid, mp 190.1–191.5 °C; ^{1}H NMR (DMSO-d_6) δ: 2.11 (s, 3H, CH_3), 7.46 (d, 1H, J = 5.2 Hz, C*H*), 7.13–7.57 (m, 8H, Ph*H*), 8.61 (d, 1H, J = 5.2 Hz, C*H*), 10.16 (s, 1H, N*H*), 12.18 (s, 1H, NO*H*); ^{13}C NMR (DMSO-d_6) δ: 19.8, 102.5, 109.2, 118.5 (2C), 120.0, 125.9, 128.8 (2C), 130.0, 133.0 (2C), 133.7, 136.1, 145.2, 155.8, 159.2, 159.6, 162.6; MS (EI) m/z 329.1 (M^+), calcd for $C_{19}H_{15}N_5O$ 329.4.

5.1.15. 4-(4-((Hydroxyimino)(*m*-tolyl)methyl)pyrimidin-2-ylamino)benzonitrile (12l)

Yield 40.2%; light yellow solid, mp 198.5–200.4 °C; ^{1}H NMR (DMSO-d_6) δ: 2.36 (s, 3H, CH_3), 7.38 (d, 1H, J = 5.2 Hz, C*H*), 7.14–7.68 (m, 8H, Ph*H*), 8.59 (d, 1H, J = 5.2 Hz, C*H*), 10.15 (s, 1H, N*H*), 12.11 (s, 1H, NO*H*); ^{13}C NMR (DMSO-d_6) δ: 21.5, 102.6, 110.1, 118.6 (2C), 120.0, 126.8, 128.3, 129.5, 130.0, 132.5, 133.0 (2C), 137.5, 145.3, 155.2, 159.0, 159.5, 163.3; MS (EI) m/z 329.1 (M^+), calcd for $C_{19}H_{15}N_5O$ 329.4.

5.1.16. 4-(4-((Hydroxyimino)(*p*-tolyl)methyl)pyrimidin-2-ylamino)benzonitrile (12m)

Yield 32.0%; light yellow solid, mp 236.5–238.2 °C; ^{1}H NMR (DMSO-d_6) δ: 2.42 (s, 3H, CH_3), 7.38 (d, 1H, J = 5.2 Hz, C*H*), 7.26–7.72 (m, 8H, Ph*H*), 8.59 (d, 1H, J = 5.2 Hz, C*H*), 10.13 (s, 1H, N*H*), 12.09 (s, 1H, NO*H*); ^{13}C NMR (DMSO-d_6) δ: 21.5, 102.7, 110.4, 118.7 (2C), 120.0, 128.9 (2C), 129.4 (2C), 129.8, 130.0 (2C), 138.4, 145.2, 154.9, 159.0, 159.5, 163.5; MS (EI) m/z 329.1 (M^+), calcd for $C_{19}H_{15}N_5O$ 329.4.

5.1.17. 4-(4-((Hydroxyimino)(3-methoxyphenyl)methyl) pyrimidin-2-ylamino) benzonitrile (12n)

Yield 27.9%; light yellow solid, mp 213.7–214.4 °C; ^{1}H NMR (DMSO-d_6) δ: 2.51 (s, 3H, OCH_3), 7.39 (d, 1H, J = 5.2 Hz, C*H*), 6.90–7.70 (m, 8H, Ph*H*), 8.59 (d, 1H, J = 5.2 Hz, C*H*), 10.15 (s, 1H, N*H*), 12.16 (s, 1H, NO*H*); ^{13}C NMR (DMSO-d_6) δ: 55.6, 102.6, 110.0, 114.4, 115.3, 118.7 (2C), 120.0, 122.0, 129.6, 133.0 (2C), 133.8, 145.2, 154.9, 159.0, 159.4, 159.5, 163.1; MS (EI) m/z 345.1 (M^+), calcd for $C_{19}H_{15}N_5O_2$ 345.4.

5.1.18. 4-(4-((Hydroxyimino)(4-methoxyphenyl)methyl) pyrimidin-2-ylamino) benzonitrile (12o)

Yield 35.4%; yellow solid, mp 185.2–187.8 °C; ^{1}H NMR (DMSO-d_6) δ: 3.78 (s, 3H, OCH_3), 7.40 (d, 1H, J = 5.2 Hz, C*H*), 6.93–7.71 (m, 8H, Ph*H*), 8.60 (d, 1H, J = 5.2 Hz, C*H*), 10.17 (s, 1H, N*H*), 12.18 (s, 1H,

NO*H*); ^{13}C NMR (DMSO-d_6) δ: 55.7, 102.7, 110.8, 113.7 (2C), 118.7 (2C), 120.0, 124.2, 131.6 (2C), 133.1 (2C), 145.3, 154.4, 158.9, 159.5, 159.8, 163.8; MS (EI) *m/z* 345.2 (M^+), calcd for $C_{19}H_{15}N_5O_2$ 345.4.

5.1.19. 4-(4-((4-*tert*-Butylphenyl)(hydroxyimino)methyl) pyrimidin-2-ylamino) benzonitrile (12p)

Yield 38.1%; light yellow solid, mp 192.3–193.2 °C; ^{1}H NMR (DMSO-d_6) δ: 1.04 (s, 9H, C*H*$_3$), 7.36 (d, 1H, *J* = 5.2 Hz, C*H*), 7.29–7.69 (m, 8H, Ph*H*), 8.57 (d, 1H, *J* = 5.2 Hz, C*H*), 10.13 (s, 1H, N*H*), 12.09 (s, 1H, NO*H*); ^{13}C NMR (DMSO-d_6) δ: 31.6 (3C), 35.0, 102.5, 110.4, 118.8 (2C), 119.9, 125.1 (2C), 129.4 (2C), 129.7, 133.0 (2C), 145.2, 151.3, 154.8, 159.0, 159.5, 163.4; MS (EI) *m/z* 371.2 (M^+), calcd for $C_{22}H_{21}N_5O$ 371.4.

5.1.20. 4-(4-((3,5-Dimethylphenyl)(hydroxyimino)methyl) pyrimidin-2-ylamino) benzonitrile (12q)

Yield 24.3%; light yellow solid, mp 209.8–210.2 °C; ^{1}H NMR (DMSO-d_6) δ: 2.33 (s, 6H, C*H*$_3$), 7.37 (d, 1H, *J* = 5.2 Hz, C*H*), 6.96–7.71 (m, 7H, Ph*H*), 8.60 (d, 1H, *J* = 5.2 Hz, C*H*), 10.16 (s, 1H, N*H*), 12.09 (s, 1H, NO*H*); ^{13}C NMR (DMSO-d_6) δ: 21.4 (2C), 102.7, 110.2, 118.6 (2C), 120.0, 127.2 (2C), 130.2, 132.5, 133.0 (2C), 137.4 (2C), 145.3, 155.2, 159.0, 159.5, 163.4; MS (EI) *m/z* 343.2 (M^+), calcd for $C_{20}H_{17}N_5O$ 343.4.

5.1.21. 4-(4-((Hydroxyimino)(naphthalen-1-yl)methyl)pyrimidin-2-ylamino) benzonitrile (12r)

Yield 34.8%; light yellow solid, mp 220.2–222.1 °C; ^{1}H NMR (DMSO-d_6) δ: 7.57 (d, 1H, *J* = 5.2 Hz, C*H*), 7.19–8.13 (m, 11H, Ph*H*, Naph*H*), 8.64 (d, 1H, *J* = 5.2 Hz, C*H*), 10.07 (s, 1H, N*H*), 12.28 (s, 1H, NO*H*); ^{13}C NMR (DMSO-d_6) δ: 102.4, 109.3, 118.3 (2C), 120.0, 125.8, 125.9, 126.5, 126.9 (2C), 128.8, 128.9, 130.7, 131.8, 132.7 (2C), 133.5, 145.0, 154.9, 159.3, 159.5,162.95; MS (EI) *m/z* 365.2 (M^+), calcd for $C_{22}H_{15}N_5O$ 365.4.

5.1.22. 4-(4-((Hydroxyimino)(naphthalen-2-yl)methyl) pyrimidin-2-ylamino) benzonitrile (12s)

Yield 21%; white solid, mp 189.5–191.0 °C; ^{1}H NMR (DMSO-d_6) δ: 7.50 (d, 1H, *J* = 5.2 Hz, C*H*), 7.08–8.08 (m, 11H, Ph*H*, Naph*H*), 8.65 (d, 1H, *J* = 5.2 Hz, C*H*), 10.14 (s, 1H, N*H*), 12.29 (s, 1H, NO*H*); ^{13}C NMR (DMSO-d_6) δ: 102.5, 110.1, 118.6 (2C), 119.9, 126.7, 127.1, 127.5, 127.7, 128.1, 128.7, 129.1, 130.3, 132.8, 133.0 (2C), 133.1, 145.1, 155.2, 159.1, 159.5, 163.2; MS (EI) *m/z* 365.1 (M^+), calcd for $C_{22}H_{15}N_5O$ 365.4.

5.2. Anti-HIV activity assays

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.[13,14] Briefly, virus stocks were titrated in MT-4 cells and expressed as 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 brought into each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. The test compounds were dissolved in DMSO at 50 mM or higher. After a 4-day 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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