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Design and synthesis of a new series of cyclopropylamino-linking diarylpyrimidines as HIV non-nucleoside reverse transcriptase inhibitors

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ABSTRACT

A new series of 29 diarylpyrimidine analogues featuring a cyclopropylamino group between the pyrimidine scaffold and the aryl wing have been synthesized. All of the new compounds have been characterized by spectra analysis. The target molecules were evaluated for their *in vitro* anti-HIV activity with FDA-approved drugs as references. Some of the compounds exhibited moderate to potent activities against wild-type HIV-1. The compound 4-((4-((cyclopropylamino)(2,5-difluorophenyl)methyl)pyrimidin-2-yl)amino)benzonitrile (**1e**) displayed potent anti-HIV-1 activity against WT HIV-1 with an IC_{50} of 0.099 μM and a selectivity index of 2302. The preliminary structure–activity relationship (SAR) of this new series of compounds was also investigated.

1. Introduction

HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTI) are the key drugs in efficient treatment of AIDS (de Béthune, 2010; Zhan et al., 2009). Diarylpyrimidine (DAPY) derivatives are one of the most successful families of NNRTIs developed so far (Chen et al., 2011), which have been confirmed by the approval of Etravirine (**2**, Fig. 1) and Rilpivirine (**3**, Fig. 1) for clinical use (Mordant et al., 2007).

To identify more potent DAPYs as possible NNRTIs (Meng et al., 2003; Meng et al., 2005), we have made considerable efforts and structural modifications on the linker of CH_2-DAPYs, which resulted in a variety of groups between the left benzene ring and the central pyrimidine ring (Feng et al., 2010; Zeng et al., 2010). The introductions of these groups, especially hydrazine group in candidate **6** or hydroxyl group in compound **7** (Gu et al., 2011, 2012) (Fig. 2) (Ma et al., 2011), were proved to be very beneficial for the improvement in antiviral activity (Ma et al., 2011). Although the docking results showed that these groups could fill the Val179-including active binding pocket of HIV-1 RT (Das et al., 2008) and generate suitable electrostatic interactions with the amino acid residues at the wall of the active site, this preliminary CADD result still needed more experimental supporting. Therefore, we aimed at more conclusive evidence that the substitution position would be effectively beneficial for the binding between the inhibitor and the NNBP of RT.

On the other hand, while considering the fact that the chemical structures of two FDA approved NNRTIs used in HIV therapy shared the same cyclopropyl group (**4**, **5**, Fig. 1), we envisaged that the introduction of a cyclopropylamino (Cpa) group might be better accommodated in the appropriated position of the NNBPs in HIV-1 RT (Hassam et al., 2012). This improvement in the steric interactions between the ligand and the active binding pocket might be an efficient strategy to design more suitable drug candidates according to the already known hydrophobic nature of the HIV-1 RT binding pocket (Antunes et al., 2011; Das et al., 2008; Hassam et al., 2012). We describe the design and synthesis of a new series of 4-(cyclopropylamino)aryl methyl DAPYs derivatives (**1**, Fig. 2) in this paper. The biological activity of all the compounds was also evaluated to delineate the structure activity relationship (SAR).

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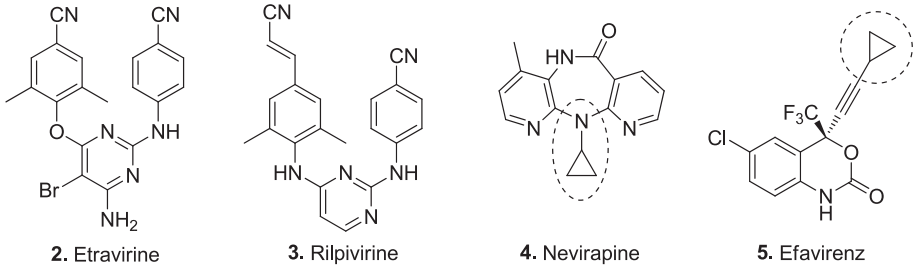


Fig. 1. Structures of some currently FDA-approved HIV-1 NNRTIs.

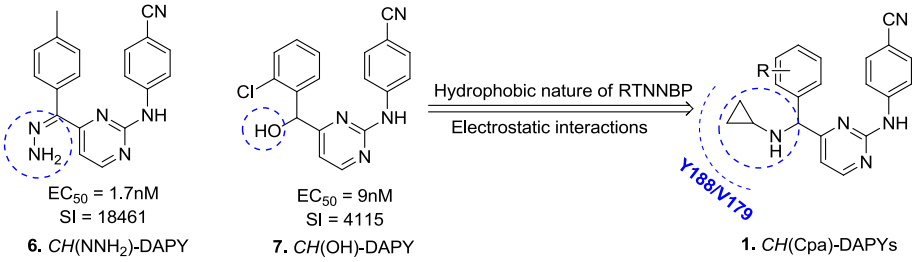
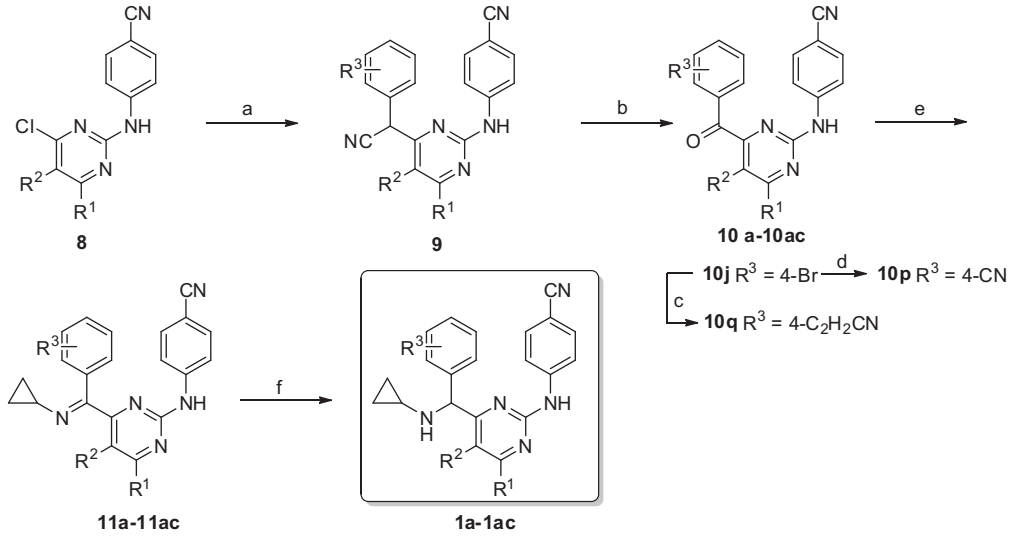


Fig. 2. Molecular design of CH(Cpa)-DAPYs.



10, 11, 1	R^1	R^2	R^3
a	H	H	4-H
b	H	H	4-Me
c	H	H	4-F
d	H	H	3-F
e	H	H	2,5-diF
f	H	H	4-Cl
g	H	H	3-Cl
h	H	H	2-Cl
i	H	H	2,4-diCl
j	H	H	4-Br

10, 11, 1	R^1	R^2	R^3
k	H	H	3-Br
l	H	H	4-*t*Bu
m	H	H	4-OMe
n	H	H	2,3-CH=CH-CH=CH-
o	H	H	3,4-CH=CH-CH=CH-
p	H	H	4-CN
q	H	H	4-C_2H_4CN
r	Me	H	4-H
s	Me	H	4-F
t	Me	H	4-Cl

10, 11, 1	R^1	R^2	R^3
u	Me	H	4-Br
v	Me	H	4-*t*Bu
w	Me	H	4-OMe
x	H	Me	4-H
y	H	Me	4-F
z	H	Me	4-Cl
aa	H	Me	4-Br
ab	H	Me	4-*t*Bu
ac	H	Me	4-OMe

Scheme 1. Synthetic route to CH(Cpa)-DAPYs (**1a–1ac**). Reagents and conditions: (a) R^3-Phenylacetonitrile, 60% NaH, Ar, DMF, -20 °C to r. t., 48–72 h; (b) NaH, air, DMF, r. t., 36–72 h; (c) CuCN, DMF, 150 °C, 10 h; (d) acrylonitrile, $Pd(OAc)_2$, P(*o*-Tol)$_3$, NaOAc, DMA, 150 °C, overnight; (e) Cyclopropylamine, EtOH, reflux, 4–8 h; and (f) $NaBH_3CN$, EtOH, 60 °C, 4 h.

2. Results and discussion

2.1. Chemistry

The synthesis of the target compounds **1a–1ac** is shown in Scheme 1. The key intermediates, oxo-CH_2-DAPYs (**10a–10ac**) were conveniently synthesized from 4-(4-chloro pyrimidin-2-ylamino)benzonitriles (**8**) through two steps of reactions, *via* the modified procedures of our previously reported methods (Hassam et al., 2012). Cyanidation of **10j** with CuCN at 150 °C for 10 h in anhydrous DMF gave the corresponding 4-cyano intermediate **10p**. The acrylonitrile derivative **10q** was prepared according to the coupling reaction which was conducted on **10j** with classical Heck conditions using palladium (II) diacetate as the catalyst in the presence of sodium acetate in DMA. Imino compounds (**11a–11ac**) were synthesized by refluxing **10a–10ac** with cyclopropylamine in ethanol with the assistance of acetic acid and anhydrous sodium sulfate. Reductions of **11a–11ac** with sodium cyanoborohydride in ethanol solution provided the final compounds **1a–1ac**.

2.2. Biological activity

The anti-HIV activity of 4-(cyclopropylamino)arylmethyl DAPYs was measured using the MTT method and compared to five FDA-approved drugs: Nevirapine, Zidovudine, Efavirenz, Delavirdine and Etravirine. The cells were infected with HIV-1 wild-type virus (LAI) or double RT mutant (K103N/Y181C) strain derived from wild-type LAI, and HIV-2 strain (ROD). The results are reported as half maximal (50%) inhibitory concentration (IC_{50}). Moreover, the cytotoxicities (CC_{50}) of the compounds were also determined. The selective index ($SI = CC_{50}/IC_{50}$), which indicates the specificity of the antiviral effect, was also given for the biological data within the wild-type HIV strain.

The biological testing results of these target DAPY analogues together with the relative data of the five reference compounds are listed in Table 1. Some of the target compounds showed moderate to potent activities against wild-type (WT) HIV-1 with IC_{50} values in the range of 9.70–0.099 μM. In this series, the compounds containing the 2,5-difluoro, 2-chloro and 4-cyanovinyl on the left phenyl ring (**1e**, **1h** and **1q**) displayed good anti-HIV-1 activity against WT HIV-1 and showed excellent selectivity. Unfortunately, none of them exhibited activity against the double RT mutant virus (K103N + Y181C) and HIV-2 strain ROD.

All of the unsubstituted parent compounds (**1a**, **1r** and **1x**) demonstrated good biological activity against HIV-1. With the introduction of a methyl group in the *para*-position of the phenyl ring, the biological activity of these compounds were kept almost unchanged. The replacement of the methyl group in these compounds by an electron-withdrawing group such as a halogen atom (**1c**, **1f** and **1j**) or a cyano group (**1p**) might result in a reduced biological potency. Replacement of the cyano substituent by an acrylonitrile group (**1q**) could lead to a very potent compound highly active against single mutant strains and with a elevated selectivity. This result provide a hint for the future research that a favorable *para*-substituent on the A-ring might be flexible in conformation and more linear in shape in order to insert into the narrow tunnel of the HIV-1 NNRTIs binding site. Introduction of a methoxy or a

Table 1
Anti-HIV activities and cytotoxicities of compounds **1a–1ac** MT-4 cells.

Compounds	IC_{50} (μM)			CC_{50} (μM)	SI
	IIIB[a]	RES056[b]	ROD		
1a	1.83 ± 0.58	>40.54	>40.54	40.54 ± 4.52	22
1b	3.68 ± 1.50	>96.95	>96.95	96.95 ± 68.50	26
1c	>33.18	>33.18	>33.18	33.18 ± 1.99	<1
1d	0.91 ± 0.34	>105.79	>105.79	105.79 ± 67.89	116
1e	0.099 ± 0.038	>228.941	>228.941	228.941 ± 58.061	2302
1f	>33.52	ND	>33.52	33.52 ± 1.32	<1
1g	1.14 ± 0.27	>101.74	>101.74	101.74 ± 31.31	89
1h	0.12 ± 0.01	>37.89	>37.89	37.89 ± 1.98	317
1i	1.08 ± 0.20	>31.39	>31.39	31.39 ± 0.95	29
1j	>28.73	ND	>28.73	28.73 ± 1.05	<1
1k	1.102 ± 0.15	>35.55	>35.55	35.55 ± 1.11	32
1l	>28.68	ND	>28.68	28.68 ± 1.03	<1
1m	>38.84	ND	>38.84	38.84 ± 2.78	<1
1n	4.66 ± 0.89	>182.19	>182.19	182.19 ± 10.59	39
1o	>29.06	ND	>29.06	29.06 ± 2.50	<1
1p	>34.71	>34.71	>34.71	34.71 ± 1.08	<1
1q	0.196 ± 0.03	>56.108	>56.108	56.108 ± 9.788	287
1r	9.69 ± 2.67	>315.10	>315.10	⩾315.10	⩾33
1s	>37.96	ND	>37.96	37.96 ± 0.83	<1
1t	>261.79	ND	>261.79	261.79 ± 70.00	<1
1u	>267.08	>267.08	>267.08	⩾267.08	<1
1v	>29.22	ND	>29.22	29.22 ± 0.50	<1
1w	>36.81	ND	>36.81	36.81 ± 0.68	<1
1x	2.05 ± 0.32	>351.68	>351.68	>351.68	>172
1y	>5.49	ND	>5.49	5.49 ± 0.69	<1
1z	>1.19	ND	>1.19	1.19 ± 0.11	<1
1aa	>2.13	ND	>2.13	2.13 ± 1.20	<1
1ab	9.70 ± 2.66	>28.96	>28.96	28.96 ± 1.14	3
1ac	>2.80	ND	>2.80	2.80 ± 1.64	<1
Nevirapine	0.101 ± 0.015	0.90		>15.021	>148
Zidovudine	3.89 ± 2.89		15.53 ± 11.17	>87.24	>22
Efavirenz	0.0066 ± 0.0006	0.0077	0.0017	>93.5489	>14,124
Delavirdine	0.0226	0.16		6.3356	>280
Etravirine	0.81			43.84	>54

[a] IIIB means the wild type of HIV-1 virus strain.
[b] ES056 means the double mutant (K103N/Y181C) HIV-1 virus strain.

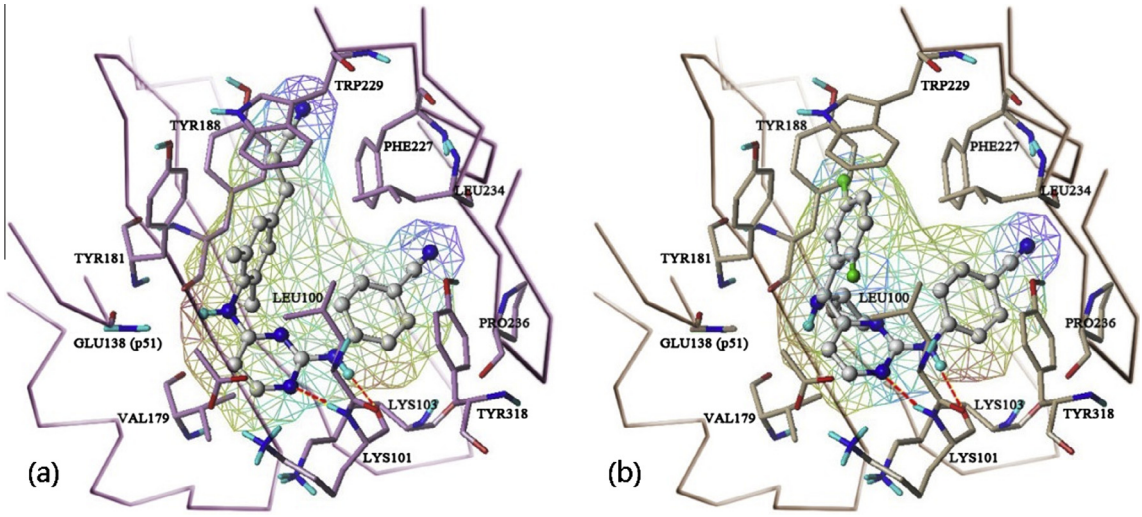


Fig. 3. Comparison of the binding modes between the representative potential compound **1e** and TMC278 in NNRTBP of HIV-1 RT (PDB: 2ZD1(Das et al., 2008)). (a) TMC278/RT^{WT}, (b) **1e**/RT^{WT}. The figures are generated by the software of SYBYL-X 2.0. Possible hydrogen bonds are indicated with dashed lines in red. The amino acid residues are shown in liner frame and the docked conformations of the small molecules are shown in ball and stick both for **1e** and TMC278, respectively. (For interpretation of the references to color in these figures legend, the reader is referred to the web version of this article.)

tert-butyl group at *para*-position could not exert any effects on the activities, which could be explained by the presence of the steric hindrance effect generated by the bulky substituents.

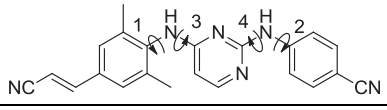
It could be deduced from the obtained biological data that the *meta* and *ortho* substitutions might lead the corresponding compounds to exhibit good potency against wild-type virus, especially for the *ortho* position. The introduction of chlorine or fluorine atoms onto the *meta* and *ortho* position resulted in compounds **1e**, **1h** with excellent activity of 0.099 μM and 0.12 μM, respectively. Additionally, **11e** was also the best one with highest selectivity (SI = 2302) and lowest cytotoxicity (CC_{50} = 228.9) out of all the active compounds. This was partially due to the presence of the groups at the *ortho* position, limiting the rotational freedom of the phenyl ring. Removal of these substituents might increase the conformational flexibility and therefore decrease the π–π interaction with the HIV-1 RT NNBP. In addition, the halogen atoms were also involved in hydrophobic interactions with the side chains of amino residues exposed on the surface of this allosteric site.

With the aim to enhance the π–π stacking interactions with the NNBP, 1-naphthyl- or 2-naphthyl-substituted parent compounds (**1n** and **1p)** were synthesized and also assayed. The results indicated that only 1-naphthyl substituted compound **1n** was determined to have IC_{50} value of 4.66 μM, while the 2-naphthyl substituted **1p** exhibited no activity against HIV-1. The decrease in biological activity might be explained by both the protruding effect and the stereospecific blockade formed between the large 2-naphthyl and the large cyclopropyl group, which therefore might cause further weakening of the π–π interactions. This similar result about the different effects between the 1-naphthyl- and the 2-naphthyl-substitutions on the biological activities against the HIV were also agreed with our previous discoveries in the naphthyl substituted HEPT analogues as the potential NNRTIs (Feng et al., 2009; Zeng et al., 2010).

The introduction of the methyl substituent in C-5 or C-6 position of the pyrimidine ring clearly resulted in unfavorable activity against most of virus strains ever tested. In these series, none of the substituted phenyl compounds exhibited activity except that the non-substituted parent compounds **1r** and **1x** displayed weak potency. Moreover, the compounds **1y**–**1ac** with a methyl group at the C-6 position of the pyrimidine also exhibited very high toxicity.

Although the introduction of the cyclopropylamino group to the methylene linker did not bring the excellent result which we had expected at first, the relating research result still might provide some deep insight of the relative SAR analysis. Compared with the hydrazine (Das et al., 2008) or hydroxyl (Ma et al., 2011) groups that we used in our previous research work, cyclopropylamino group brought more steric hindrance to the neighboring groups. This bulky group maybe liable to destroy the proper binding orientation position between the compounds and the NNRTI binding site, which have been called as the horseshoe conformational shape (Gu et al., 2011). Superposition of all the compounds using the conformations after docking (Jain, 2009) in the NNRTI pocket showed that an unfavorable connection appeared in some compounds with lower activities, in which the cyclopropyl group was protruding into the binding pocket instead of the phenyl ring A (Fig. 3) (Spitzer and Jain, 2012). Because the previous relative SAR studies have showed that π–π main interactions were present between the phenyl ring A of the ligands and residues Tyr181 and Tyr188 in the allosteric binding pocket (Tian et al., 2010), a cyclopropyl might therefore weaken the π–π interaction (Thakur et al., 2008). With this point of view, it is not surprising that all the new compounds exhibit relatively lower activities against HIV-1 virus

Table 2
Comparison of the torsion angles of the binding conformations of TMC278 and compound **1e**.

	Compound-RT complex	Torsion angles (°)			
		τ1	τ2	τ3	τ4
	TMC278/RT^{WT}	85.0	16.1	−11.5	−12.0
	1e/RT^{WT}	−35.1	28.0	−48.9	0.6

compared with other FDA-approved drugs. The comparisons between the torsion angles of the active binding conformations of both **1e** with RT^{WT} and TMC278-RT cocrystal structure (Das et al., 2008) could also explain the biological difference of compound **1e** from that of TMC278 (Table 2).

3. Conclusions

A series of 4-(cyclopropylamino)aryl methyl DAPYs were successfully synthesized *via* several steps as part of our anti-HIV program. Biological evaluation indicated that the newly synthesized derivatives showed moderate to potent activities against wild-type (WT) HIV-1. The exploratory study clearly enriched the SAR of CH_2-DAPYs as anti-HIV agents. Compounds **1e** displayed good anti-HIV-1 activity against WT HIV-1 with an IC_{50} value of 0.099 μM and a selectivity index of 2302. None of the compounds exhibited activity against the mutant virus. Therefore, this study clearly highlighted the necessary to keep a proper volume of the substituent group to the CH_2-linker and a satisfactory flexibility of the whole molecule to achieve a higher level of inhibition on NNRTI-resistant viruses. Furthermore, the result has enriched the SAR of DAPY analogues as potent anti-HIV-1 compounds. These conclusions could provide valuable information for the rational design of effective inhibitors with better therapeutic profiles against AIDS.

4. Experimental section

4.1. Chemistry

Chemical reagents and solvents, purchased from commercial sources, were of analytical grade and were used without further purification. Melting points were measured on a SGW X-1 microscopic melting point apparatus. TLC analyses were run on pre-coated silica gel G plates at 254 nm under a UV lamp using a variety of solvent systems. Column chromatography separations were performed with silica gel (300–400 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV400 MHz spectrometer 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 electron spray ionization (ESI) techniques.

4.1.1. Procedure for the synthesis of ***10p***

A mixture of **10j** (756 mg, 2.00 mmol), CuCN (178 mg, 2.00 mmol) and DMF (2.00 mL) was heated at 150 °C for 10 h with stirring, then cooled. The suspension was a solution of iron (III) chloride (1.00 g), concentrated HCl (0.20 mL), and water (20.0 mL). The mixture was stirred at 70 °C for 20 min and extracted with CH_2Cl_2 (20.0 mL × 3). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (silica gel; CH_2Cl_2) gave **10p**.

4.1.2. Procedures for the synthesis of ***10q***

A mixture of **10j** (756 mg, 2.00 mmol), acrylonitrile (1.06 g, 20.0 mmol), $Pd(OAc)_2$ (89.9 mg, 0.40 mmol), tri-*o*-tolyl phosphine (608 mg, 2.00 mmol) and sodium acetate (656 mg, 8.00 mmol) in anhydrous DMA (30.0 mL) was stirred in a sealed tube at 150 °C overnight. The reaction mixture was then hydrolyzed with water and extracted with CH_2Cl_2 (20.0 mL × 3). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product **10q** was recrystallized from acetonitrile to afford the pure product, which could be used directly in the next step.

4.1.3. General procedures for the synthesis of target compounds ***1a–1ac***

To a mixture of **10a–1ac** (1.00 mmol), Na_2SO_4 (213 mg, 1.50 mmol) and ethanol (20.0 mL) was added cyclopropyl-amine (285 mg, 5.00 mmol) and 5 drops of acetic acid. The mixture was heated to reflux for 4–8 h until the reaction completed which was monitored by TLC, then cooled. Sodium cyanoborohydride was added at room temperature and subsequently the mixture was heated at 60 °C for 4 h, then poured into saturated sodium bicarbonate and extracted with CH_2Cl_2 (20.0 mL × 3). The organic layers were combined, dried over anhydrous $MgSO_4$, and was concentrated under reduced pressure. Next, the residue was purified by column chromatography (silica gel; EtOAc/petroleum ether, 1:5–1:3).

4-((4-((Cyclopropylamino)(phenyl)methyl)pyrimidin-2-yl)amino)benzonitrile (1a) Yield: 58%; mp 149.8–151.8 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.15 (s, 1H, NH), 8.49 (d, J = 5.1 Hz, 1H, pyrimidine H_6), 7.94 (d, J = 8.7 Hz, 2H, Ar$H_{3,5}$), 7.69 (d, J = 8.7 Hz, 2H, Ar$H_{2,6}$), 7.42 (d, J = 7.4 Hz, 2H, Ar′$H_{2,6}$), 7.32 (t, J = 7.5 Hz, 2H, Ar′$H_{3,5}$), 7.24 (d, J = 7.2 Hz, 1H, Ar′H_4), 7.15 (d, J = 5.1 Hz, 1H, pyrimidine H_5), 4.81 (s, 1H, CH), 2.04–1.91 (m, 1H, CH), 0.39–0.29 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 173.14, 159.51, 158.80, 145.42, 142.57, 133.43 (2C), 128.80 (2C), 127.96 (2C), 127.62, 120.07, 118.71 (2C), 111.61, 102.68, 67.87, 29.37, 6.81 (2C); MS (ESI+) m/z 342 $(M+H)^+$; HR-MS: m/z Calcd for $C_{21}H_{19}N_5$: 341.1640. Found: 341.1649.

4-((4-((Cyclopropylamino)(p-tolyl)methyl)pyrimidin-2-yl)amino)benzonitrile (1b) Yield: 62%; mp 177.8–179.4 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.14 (s, 1H, NH), 8.48 (d, J = 5.1 Hz, 1H, pyrimidine H_6), 7.95 (d, J = 8.7 Hz, 2H, Ar$H_{3,5}$), 7.69 (d, J = 8.7 Hz, 2H, Ar$H_{2,6}$), 7.30 (d, J = 7.9 Hz, 2H, Ar′$H_{2,6}$), 7.21–7.05 (m, 3H, Ar′$H_{3,5}$ + -pyrimidine H_5), 4.77 (s, 1H, CH), 2.24 (s, 3H, CH_3), 2.02–1.91 (m, 1H, CH), 0.41–0.23 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 173.31, 159.49, 158.71, 145.44, 139.59, 136.73, 133.43 (2C), 129.35 (2C), 127.82 (2C), 120.08, 118.70 (2C), 111.54, 102.66, 67.60, 29.35, 21.10, 6.78 (2C); MS (ESI+) m/z 356 $(M+H)^+$; HR-MS: m/z Calcd for $C_{22}H_{21}N_5$: 355.1797. Found: 355.1806.

4-((4-((Cyclopropylamino)(4-fluorophenyl)methyl)pyrimidin-2-yl)amino)benzonitrile (1c) Yield: 48%; mp 157.9–159.1 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.16 (s, 1H, NH), 8.50 (d, J = 5.0 Hz, 1H, pyrimidine H_6), 7.93 (d, J = 8.5 Hz, 2H, Ar$H_{3,5}$), 7.69 (d, J = 8.5 Hz, 2H, Ar$H_{2,6}$), 7.59–7.39 (m, 2H, 2H, Ar′$H_{2,6}$), 7.32–7.05 (m, 3H, Ar′$H_{3,5}$ + pyrimidine H_5), 4.83 (s, 1H, CH), 2.03–1.92 (m, 1H, CH), 0.41–0.26 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 172.87, 161.77 (d, J_{CF} = 243.2 Hz), 159.53, 158.93, 145.38, 138.77, 133.43 (2C), 129.89 (2C, d, J_{CF} = 8.0 Hz), 120.07, 118.72 (2C), 115.50 (2C, d, J_{CF} = 21.2 Hz), 111.56, 102.72, 67.05, 29.32, 6.82, 6.77; MS (ESI+) m/z 360 $(M+H)^+$; HR-MS: m/z Calcd for $C_{21}H_{18}FN_5$: 359.1546. Found: 359.1555.

4-((4-((Cyclopropylamino)(3-fluorophenyl)methyl)pyrimidin-2-yl)amino)benzonitrile (1d) Yield: 46%; mp 160.4–161.9 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.16 (s, 1H, NH), 8.50 (d, J = 5.1 Hz, 1H, pyrimidine H_6), 7.92 (d, J = 8.8 Hz, 2H, Ar$H_{3,5}$), 7.67 (d, J = 8.8 Hz, 2H, Ar$H_{2,6}$), 7.36 (dd, J = 14.1, 7.8 Hz, 1H, Ar′H), 7.27 (t, J = 10.9 Hz, 2H, Ar′H), 7.14 (d, J = 5.1 Hz, 1H, pyrimidine H_5), 7.10–7.01 (m, 1H, Ar′H), 4.85 (s, 1H, CH), 3.52 (s, 1H, NH), 2.03–1.91 (m, 1H, CH), 0.39–0.26 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 172.46, 162.70 (d, J_{CF} = 243.5 Hz), 159.52, 159.01, 145.57 (d, J_{CF} = 6.8 Hz,) 133.40 (2C), 130.68 (d, J_{CF} = 8.2 Hz), 124.20, 120.06, 118.74 (2C), 114.68, 114.48 (d, J_{CF} = 2.3 Hz), 114.29, 111.60, 102.74, 67.24, 29.26, 6.86, 6.79; MS (ESI+) m/z 360 $(M+H)^+$; HR-MS: m/z Calcd for $C_{21}H_{18}FN_5$: 359.1546. Found: 359.1559.

4-((4-((Cyclopropylamino)(2,5-difluorophenyl)methyl)pyrimidin-2-yl)amino)benzonitrile (1e) Yield: 44%; mp 178.1–179.2 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.12 (s, 1H, NH), 8.52 (d, J = 5.0 Hz,

1H, pyrimidine H_6), 7.83 (d, J = 8.7 Hz, 2H, Ar$H_{3,5}$), 7.62 (d, J = 8.7 Hz, 2H, Ar$H_{2,6}$), 7.46–7.38 (m, 1H, Ar′H), 7.29–7.20 (m, J = 9.1, 4.5 Hz, 1H, Ar′H), 7.20–7.13 (m, 1H, Ar′H), 7.11 (d, J = 5.0 Hz, 1H, pyrimidine H_5), 5.10 (d, J = 8.1 Hz, 1H, CH), 3.57 (s, 1H, NH), 2.06–1.94 (m, 1H, CH), 0.45–0.27 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 171.21, 159.49, 159.24, 158.85(d, J = 242.0 Hz), 156.73 (d, J = 242.0 Hz), 145.30, 133.27 (2C), 131.62, 120.02, 118.71 (2C), 117.26 (2C, dd, J = 25.0, 8.8 Hz), 116.18–115.55 (2C, m), 111.68, 102.78, 60.57, 29.21, 7.02, 6.68; MS (ESI+) m/z 378 $(M+H)^+$; HR-MS: m/z Calcd for $C_{21}H_{17}F_2N_5$: 377.1452. Found: 377.1461.

4-((4-((4-Chlorophenyl)(cyclopropylamino)methyl)pyrimidin-2-yl)amino)benzonitrile (1f) Yield: 42%; mp 159.6–161.4 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.17 (s, 1H, NH), 8.51 (d, J = 5.1 Hz, 1H, pyrimidine H_6), 7.92 (d, J = 8.6 Hz, 2H, Ar$H_{3,5}$), 7.69 (d, J = 8.6 Hz, 2H, Ar$H_{2,6}$), 7.42 (dd, J = 22.1, 8.4 Hz, 4H, Ar′H), 7.14 (d, J = 5.1 Hz, 1H, pyrimidine H_5), 4.84 (d, J = 6.0 Hz, 1H, CH), 3.49 (s, 1H, NH), 2.02–1.92 (m, 1H, CH), 0.38–0.29 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 172.62, 159.53, 158.99, 145.37, 141.65, 133.42 (2C), 132.18, 129.90 (2C), 128.73 (2C), 120.06, 118.72 (2C), 111.58, 102.73, 67.08, 29.31, 6.77 (2C); MS (ESI+) m/z 376 $(M+H)^+$; HR-MS: m/z Calcd for $C_{21}H_{18}ClN_5$: 375.1251. Found: 375.1259.

4-((4-((3-Chlorophenyl)(cyclopropylamino)methyl)pyrimidin-2-yl)amino)benzonitrile (1g) Yield: 38%; mp 171.2–172.1 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.17 (s, 1H, NH), 8.52 (d, J = 5.1 Hz, 1H, pyrimidine H_6), 7.92 (d, J = 8.8 Hz, 2H, Ar$H_{3,5}$), 7.68 (d, J = 8.7 Hz, 2H, Ar$H_{2,6}$), 7.54 (s, 1H, Ar′H_2), 7.43–7.26 (m, 3H, Ar′$H_{4,5,6}$), 7.16 (d, J = 5.1 Hz, 1H, pyrimidine H_5), 4.84 (s, 1H, CH), 3.57 (s, 1H, NH), 2.01–1.91 (m, 1H, CH), 0.38–0.28 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 172.39, 159.53, 159.09, 145.36, 145.20, 133.51, 133.40 (2C), 130.66, 127.78, 127.58, 126.86, 120.06, 118.75 (2C), 111.59, 102.74, 67.20, 29.26, 6.90, 6.79; MS (ESI+) m/z 376 $(M+H)^+$; HR-MS: m/z Calcd for $C_{21}H_{18}ClN_5$: 375.1251. Found: 375.1261.

4-((4-((2-Chlorophenyl)(cyclopropylamino)methyl)pyrimidin-2-yl)amino)benzonitrile (1h) Yield: 21%; mp 169.6–171.4 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.13 (s, 1H, NH), 8.53 (d, J = 5.0 Hz, 1H, pyrimidine H_6), 7.84 (d, J = 8.7 Hz, 2H, Ar$H_{3,5}$), 7.62 (d, J = 8.7 Hz, 2H, Ar$H_{2,6}$), 7.57 (d, J = 7.1 Hz, 1H, Ar′H3), 7.51–7.24 (m, 3H, Ar′H4,5,6), 7.11 (d, J = 5.1 Hz, 1H, pyrimidine H_5), 5.28 (d, J = 8.6 Hz, 1H, CH), 3.54 (d, J = 8.8 Hz, 1H, NH), 2.06–1.97 (m, 1H, CH), 0.56–0.20 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 171.54, 159.51, 159.07, 145.33, 139.89, 133.53, 133.29 (2C), 129.90, 129.74, 129.29, 127.80, 120.05, 118.67 (2C), 112.03, 102.66, 63.92, 29.35, 7.04, 6.86; MS (ESI+) m/z 376 $(M+H)^+$; HR-MS: m/z Calcd for $C_{21}H_{18}ClN_5$: 375.1251. Found: 375.1265.

4-((4-((Cyclopropylamino)(2,4-dichlorophenyl)methyl)pyrimidin-2-yl)amino)benzonitrile (1i) Yield: 33%; mp 139.7–141.1 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.15 (s, 1H, NH), 8.54 (d, J = 5.0 Hz, 1H, pyrimidine H_6), 7.81 (d, J = 8.6 Hz, 2H, Ar$H_{3,5}$), 7.74–7.56 (m, 4H, Ar$H_{2,6}$ + Ar′$H_{3,5}$), 7.50 (d, J = 6.8 Hz, 1H, Ar′H_6), 7.11 (d, J = 5.0 Hz, 1H, pyrimidine H_5), 5.25 (d, J = 9.0 Hz, 1H, CH), 3.61 (d, J = 8.6 Hz, 1H, NH), 2.07–1.93 (m, 1H, CH), 0.56–0.19 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 171.01, 159.49, 159.21, 145.29, 139.20, 134.48, 133.27 (2C), 132.89, 131.37, 129.11, 127.99, 120.04, 118.68 (2C), 112.05, 102.71, 63.58, 29.32, 7.12, 6.79; MS (ESI+) m/z 410 $(M+H)^+$; HR-MS: m/z Calcd for $C_{21}H_{17}Cl_2N_5$: 409.0861. Found: 409.0869.

4-((4-((4-Bromophenyl)(cyclopropylamino)methyl)pyrimidin-2-yl)amino)benzonitrile (1j) Yield: 42%; mp 179.4–180.1 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.17 (s, 1H, NH), 8.51 (d, J = 5.1 Hz, 1H, pyrimidine H_6), 7.92 (d, J = 8.8 Hz, 2H, Ar$H_{3,5}$), 7.69 (d, J = 8.8 Hz, 2H, Ar$H_{2,6}$), 7.53 (d, J = 8.4 Hz, 2H, Ar′$H_{2,6}$), 7.39 (d, J = 8.4 Hz, 2H, Ar′$H_{3,5}$), 7.13 (d, J = 5.1 Hz, 1H, pyrimidine H_5), 4.82 (d, J = 8.3 Hz, 1H, CH), 3.48 (d, J = 7.0 Hz, 1H, NH), 2.01–1.92 (m, CH), 0.39–0.27 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 172.55, 159.52, 158.99, 145.37, 142.08, 133.42 (2C), 131.65 (2C), 130.28 (2C), 120.72, 120.06, 118.72 (2C), 111.58, 102.73, 67.14, 29.31, 6.90, 6.77; MS (ESI+) m/z 420 $(M+H)^+$; HR-MS: m/z Calcd for $C_{21}H_{18}BrN_5$: 419.0746. Found: 419.0751.

4-((4-((3-Bromophenyl)(cyclopropylamino)methyl)pyrimidin-2-yl)amino)benzonitrile (1k) Yield: 52%; mp 170.1–171.9 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.16 (s, 1H, NH), 8.51 (d, J = 5.0 Hz, 1H, pyrimidine H_6), 7.91 (d, J = 8.7 Hz, 2H, Ar$H_{3,5}$), 7.68 (d, J = 8.5 Hz, 3H, Ar$H_{2,6}$ + Ar′H_2), 7.43 (t, J = 7.7 Hz, 2H, Ar′$H_{4,6}$), 7.29 (t, J = 7.8 Hz, 1H, Ar′H_5), 7.14 (d, J = 5.1 Hz, 1H, pyrimidine H_5), 4.83 (s, 1H, CH), 3.51 (d, J = 29.4 Hz, 1H, NH), 2.01–1.89 (m, 1H, CH), 0.40–0.28 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 172.34, 159.52, 159.10, 145.43, 145.34, 133.40 (2C), 130.98, 130.65, 130.48, 127.22, 122.18, 120.06, 118.76 (2C), 111.60, 110.01, 102.74, 67.15, 29.26, 6.90, 6.77; MS (ESI+) m/z 420 $(M+H)^+$; HR-MS: m/z Calcd for $C_{21}H_{18}BrN_5$: 419.0746. Found: 419.0758.

4-((4-((4-(*tert*-Butyl)phenyl)(cyclopropylamino)methyl)pyrimidin-2-yl)amino)benzonitrile (1l) Yield: 55%; mp 139.3–141.3 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.15 (s, 1H, NH), 8.48 (d, J = 5.1 Hz, 1H, pyrimidine H_6), 7.95 (d, J = 8.7 Hz, 2H, Ar$H_{3,5}$), 7.70 (d, J = 8.6 Hz, 2H, Ar$H_{2,6}$), 7.33 (s, 4H, Ar′H), 7.15 (d, J = 5.1 Hz, 1H, pyrimidine H_5), 4.77 (s, 1H, CH), 2.01–1.91 (m, 1H, CH), 1.23 (s, 9H, *t*-Bu), 0.37–0.29 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 173.34, 159.52, 158.74, 149.96, 145.44, 139.55, 133.44 (2C), 127.54 (2C), 125.56 (2C), 120.08, 118.70 (2C), 111.56, 102.66, 67.60, 34.62, 31.58 (3C), 29.40, 6.89, 6.68; MS (ESI+) m/z 398 $(M+H)^+$; HR-MS: m/z Calcd for $C_{25}H_{27}N_5$: 397.2266. Found: 397.2275.

4-((4-((Cyclopropylamino)(4-methoxyphenyl)methyl)pyrimidin-2-yl)amino)benzonitrile (1m) Yield: 39%; mp 163.3–163.7 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.15 (s, 1H, NH), 8.48 (d, J = 5.1 Hz, 1H, pyrimidine H_6), 7.96 (d, J = 8.7 Hz, 2H, Ar$H_{3,5}$), 7.70 (d, J = 8.7 Hz, 2H, Ar$H_{2,6}$), 7.33 (d, J = 8.5 Hz, 2H, Ar′$H_{2,6}$), 7.11 (d, J = 5.1 Hz, 1H, pyrimidine H_5), 6.88 (d, J = 8.5 Hz, 2H, Ar′$H_{3,5}$), 4.76 (s, 1H, CH), 3.70 (s, 3H, OCH_3), 2.04–1.92 (m, 1H, CH), 0.42–0.24 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 173.45, 159.51, 158.85, 158.70, 145.46, 134.59, 133.43 (2C), 129.02 (2C), 120.09, 118.70 (2C), 114.18 (2C), 111.48, 102.66, 67.28, 55.51, 29.36, 6.80, 6.76; MS (ESI+) m/z 372 $(M+H)^+$; HR-MS: m/z Calcd for $C_{22}H_{21}N_5O$: 371.1746. Found: 371.1755.

4-((4-((Cyclopropylamino)(naphthalen-1-yl)methyl)pyrimidin-2-yl)amino)benzonitrile (1n) Yield: 45%; mp 173.7–175.2 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.14 (s, 1H, NH), 8.49 (d, J = 5.1 Hz, 1H, pyrimidine H_6), 8.37 (d, J = 8.4 Hz, 1H, Ar′H_8), 8.04–7.41 (m, 10H, Ar$H_{2,3,5,6+}$ Ar′$H_{2,3,4,5,6,7}$), 7.18 (d, J = 5.1 Hz, 1H, pyrimidine H_5), 5.63 (d, J = 5.3 Hz, 1H, CH), 3.56 (s, 1H, NH), 2.15–2.03 (m, 1H, CH), 0.46–0.23 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 173.02, 159.42, 158.85, 145.33, 138.41, 133.92, 133.32 (2C), 131.52, 129.11, 128.10 (2C), 126.61, 126.07, 125.99, 125.54, 124.26, 120.07, 118.64, 111.93, 102.62, 63.79, 29.74, 6.93, 6.84; MS (ESI+) m/z 392 $(M+H)^+$; HR-MS: m/z Calcd for $C_{25}H_{21}N_5$: 391.1797. Found: 391.1805.

4-((4-((Cyclopropylamino)(naphthalen-2-yl)methyl)pyrimidin-2-yl)amino)benzonitrile (1o) Yield: 40%; mp 143.1–144.3 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.17 (s, 1H, NH), 8.51 (d, J = 5.1 Hz, 1H, pyrimidine H_6), 7.99–7.42 (m, 11H, ArH + Ar′H), 7.21 (d, J = 5.1 Hz, 1H, pyrimidine H_5), 5.00 (d, J = 7.6 Hz, 1H, CH), 3.57 (d, J = 6.7 Hz, 1H, NH), 2.08–1.97 (m, 1H, CH), 0.43–0.29 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 172.94, 159.50, 158.89, 145.40, 140.20, 133.36 (2C), 133.33, 132.76, 128.37, 128.19, 127.97, 126.67, 126.63, 126.33, 126.23, 120.07, 118.68 (2C), 111.71, 111.71, 102.64, 67.97, 29.45, 6.92, 6.79; MS (ESI+) m/z 392 $(M+H)^+$; HR-MS: m/z Calcd for $C_{25}H_{21}N_5$: 391.1797. Found: 391.1809.

4-((4-((4-Cyanophenyl)(cyclopropylamino)methyl)pyrimidin-2-yl)amino)benzonitrile (1p) Yield: 38%; ^{1}H NMR (400 MHz, DMSO) δ 10.16 (s, 1H, NH), 8.51 (d, J = 5.1 Hz, 1H, pyrimidine H_6), 7.88 (d, J = 8.7 Hz, 2H, Ar$H_{3,5}$), 7.81 (d, J = 8.2 Hz, 2H, Ar$H_{2,6}$), 7.65 (dd, J = 15.9, 8.4 Hz, 4H, Ar′H), 7.14 (d, J = 5.1 Hz, 1H, pyrimidine H_5), 4.93 (s, 1H, CH), 2.01–1.90 (m, 1H, CH), 0.39–0.26 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 171.90, 159.54, 159.19, 148.28, 145.28, 133.41 (2C), 132.75 (2C), 129.13 (2C), 120.04, 119.29, 118.75 (2C), 111.71, 110.42, 102.81, 67.36, 29.28, 6.95, 6.75; MS (ESI+) m/z 367 $(M + H)^+$; HR-MS: m/z Calcd for $C_{22}H_{18}N_6$: 366.1593. Found: 366.1602.

4-((4-((4-(2-Cyanovinyl)phenyl)(cyclopropylamino)methyl) pyrimidin-2-yl)amino)benzonitrile (1q) Yield: 40%; ^{1}H NMR (400 MHz, DMSO) δ 10.14 (s, 1H, NH), 8.49 (d, J = 5.0 Hz, 1H, pyrimidine H_6), 7.90 (d, J = 8.8 Hz, 2H, Ar$H_{3,5}$), 7.67 (d, J = 8.8 Hz, 2H, Ar$H_{2,6}$), 7.64–7.56 (m, 3H, Ar′H + alkenyl H), 7.49 (d, J = 8.2 Hz, 2H, Ar′H), 7.13 (d, J = 5.1 Hz, 1H, pyrimidine H_5), 6.40 (d, J = 16.8 Hz, 1H, alkenyl H), 4.85 (s, 1H, CH), 2.02–1.91 (m, 1H, CH), 0.39–0.27 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 172.49, 159.51, 159.00, 150.74, 145.79, 145.35, 133.41 (2C), 133.24, 128.62 (2C), 128.24 (2C), 120.06, 119.31, 118.74 (2C), 111.62, 102.74, 96.96, 67.51, 29.32, 6.90, 6.75; MS (ESI+) m/z 393 $(M + H)^+$; HR-MS: m/z Calcd for $C_{24}H_{20}N_6$: 392.1749. Found: 392.1758.

4-((4-((Cyclopropylamino)(phenyl)methyl)-6-methylpyrimidin-2-yl)amino)benzonitrile (1r) Yield: 59%; mp 166.4–167.6 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.07 (s, 1H, NH), 7.94 (d, J = 8.8 Hz, 2H, Ar$H_{3,5}$), 7.67 (d, J = 8.8 Hz, 2H, Ar$H_{2,6}$), 7.42 (d, J = 7.4 Hz, 2H, Ar′$H_{2,6}$), 7.32 (t, J = 7.5 Hz, 2H, Ar′$H_{3,5}$), 7.22 (t, J = 7.3 Hz, 1H, Ar′H_4), 7.03 (s, 1H, pyrimidine H_5), 4.77 (s, 1H, CH), 2.37 (s, 3H, CH_3), 2.02–1.91 (m, 1H, CH), 0.40–0.27 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 172.66, 168.51, 159.39, 145.61, 142.71, 133.41 (2C), 128.75 (2C), 127.95 (2C), 127.54, 120.13, 118.61 (2C), 110.84, 102.45, 67.72, 29.36, 24.25, 6.82, 6.78; MS (ESI+) m/z 356 $(M + H)^+$; HR-MS: m/z Calcd for $C_{22}H_{21}N_5$: 355.1797. Found: 355.1790.

4-((4-((Cyclopropylamino)(4-fluorophenyl)methyl)-6-methylpyrimidin-2-yl)amino)benzonitrile (1s) Yield: 37%; mp 167.0–167.9 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.06 (s, 1H, NH), 7.93 (d, J = 8.7 Hz, 2H, Ar$H_{3,5}$), 7.67 (d, J = 8.7 Hz, 2H, Ar$H_{2,6}$), 7.45 (dd, J = 8.3, 5.8 Hz, 2H, Ar′$H_{2,6}$), 7.14 (t, J = 8.8 Hz, 2H, Ar′$H_{3,5}$), 7.01 (s, 1H, pyrimidine H_5), 4.79 (s, 1H, CH), 2.37 (s, 3H, CH_3), 2.00–1.91 (m, 4.4 Hz, 1H, CH), 0.39–0.26 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 172.37, 168.67, 161.74 (d, J_{CF} = 242.6 Hz), 159.41, 145.57, 138.93, 133.40 (2C), 129.88 (2C, d, J_{CF} = 8.1 Hz), 120.12, 118.63 (2C), 115.44 (2C, d, J_{CF} = 21.2 Hz), 110.82, 102.49, 66.90, 29.32, 24.24, 6.85, 6.72; MS (ESI+) m/z 374 $(M + H)^+$; HR-MS: m/z Calcd for $C_{22}H_{20}FN_5$: 373.1703. Found: 373.1712.

4-((4-((4-Chlorophenyl)(cyclopropylamino)methyl)-6-methylpyrimidin-2-yl)amino)benzonitrile (1t) Yield: 32%; mp 188.1–190.4 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.06 (s, 1H, NH), 7.91 (d, J = 8.8 Hz, 2H, Ar$H_{3,5}$), 7.66 (d, J = 8.7 Hz, 2H, Ar$H_{2,6}$), 7.41 (dd, J = 25.3, 8.4 Hz, 4H, Ar′H), 7.01 (s, 1H, pyrimidine H_5), 4.79 (s, 1H, CH), 2.37 (s, 3H, CH_3), 2.01–1.90 (m, 1H, CH), 0.38–0.27 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 172.09, 168.74, 159.39, 145.54, 141.76, 133.39 (2C), 132.12, 129.90 (2C), 128.68 (2C), 120.12, 118.64 (2C), 110.87, 102.50, 66.93, 29.31, 24.24, 6.90, 6.69; MS (ESI+) m/z 390 $(M + H)^+$; HR-MS: m/z Calcd for $C_{22}H_{20}ClN_5$: 389.1407. Found: 389.1421.

4-((4-((4-Bromophenyl)(cyclopropylamino)methyl)-6-methylpyrimidin-2-yl)amino)benzonitrile (1u) Yield: 35%; mp 193.1–194.5 °C; ^{11}H NMR (400 MHz, DMSO) δ 10.10 (s, 1H, NH), 7.92 (d, J = 8.6 Hz, 2H, Ar$H_{3,5}$), 7.67 (d, J = 8.6 Hz, 2H, Ar$H_{2,6}$), 7.53 (d, J = 8.2 Hz, 2H, Ar′$H_{2,6}$), 7.39 (d, J = 8.2 Hz, 2H, Ar′$H_{3,5}$), 7.02 (s, 1H, pyrimidine H_5), 4.78 (d, J = 7.4 Hz, 1H, CH), 3.43 (s, 1H, NH), 2.38 (s, 3H, CH_3), 0.02–1.89 (m, CH), 0.40–0.26 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 172.10, 168.68, 159.39, 145.56, 142.24, 133.40 (2C), 131.60 (2C), 130.28 (2C), 120.64, 120.12, 118.62 (2C), 110.84, 102.49, 67.02, 29.32, 24.26, 6.93, 6.73; MS (ESI+) m/z 434 $(M + H)^+$; HR-MS: m/z Calcd for $C_{22}H_{20}BrN_5$: 433.0902. Found: 433.0911.

4-((4-((4-(*tert*-Butyl)phenyl)(cyclopropylamino) methyl)-6-methylpyrimidin-2-yl)amino)benzonitrile (1v) Yield: 40%; mp 163.1–164.0 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.09 (s, 1H, NH), 7.98 (d, J = 8.7 Hz, 2H, Ar$H_{3,5}$), 7.69 (d, J = 8.7 Hz, 2H, Ar$H_{2,6}$), 7.43–7.19 (m, 4H, Ar′H), 7.05 (s, 1H, pyrimidine H_5), 4.74 (d, J = 8.4 Hz, 1H, CH), 3.28 (d, J = 6.6 Hz, 1H, NH), 2.38 (s, 3H, CH_3), 2.03–1.90 (m, 1H, CH), 1.23 (s, 9H, *t*-Bu), 0.39–0.27 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 172.91, 168.41, 159.41, 149.85, 145.66, 139.72, 133.42 (2C), 127.52 (2C), 125.51 (2C), 120.14, 118.59 (2C), 110.75, 102.43, 67.48, 34.62, 31.60 (3C), 29.39, 24.27, 6.87, 6.73; MS (ESI+) m/z 412 $(M + H)^+$; HR-MS: m/z Calcd for $C_{26}H_{29}N_5$: 411.2423. Found: 4112432.

4-((4-((Cyclopropylamino)(4-methoxyphenyl)methyl)-6-methylpyrimidin-2-yl)amino)benzonitrile (1w) Yield: 29%; mp 166.5–167.1 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.08 (s, 1H, NH), 7.97 (d, J = 8.8 Hz, 2H, Ar$H_{3,5}$), 7.68 (d, J = 8.7 Hz, 2H, Ar$H_{2,6}$), 7.33 (d, J = 8.6 Hz, 2H, Ar′$H_{2,6}$), 7.00 (s, 1H, pyrimidine H_5), 6.87 (d, J = 8.6 Hz, 2H, Ar′$H_{3,5}$), 4.72 (d, J = 8.2 Hz, 1H, CH), 3.70 (s, 3H, OCH_3), 3.26 (d, J = 6.4 Hz, 1H, NH), 2.37 (s, 3H, CH_3), 2.02–1.91 (m 1H, CH), 0.42–0.23 (m, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 172.99, 168.37, 159.40, 158.80, 145.66, 134.75, 133.41 (2C), 129.00 (2C), 120.14, 118.59 (2C), 114.13 (2C), 110.70, 102.43, 67.13, 55.50, 29.35, 24.25, 6.77 (2C); MS (ESI+) m/z 386 $(M + H)^+$; HR-MS: m/z Calcd for $C_{23}H_{23}N_5O$: 385.1903. Found: 385.1912.

4-((4-((Cyclopropylamino)(phenyl)methyl)-5-methylpyrimidin-2-yl)amino)benzonitrile (1x) Yield: 37%; mp 225.1–227.2 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.09 (s, 1H, NH), 8.31 (s, 1H, pyrimidine H_6), 7.91 (d, J = 8.7 Hz, 2H, Ar$H_{3,5}$), 7.68 (d, J = 8.6 Hz, 2H, Ar$H_{2,6}$), 7.32 (ddd, J = 36.2, 16.1, 7.1 Hz, 5H, Ar′H), 5.04 (d, J = 8.0 Hz, 1H, CH), 2.17 (s, 3H, CH_3), 1.97–1.89 (m, 1H, CH), 0.41–0.27 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 169.19, 159.21, 158.33, 145.66, 141.44, 133.47 (2C), 128.80 (2C), 128.28 (2C), 127.57, 120.51, 120.17, 118.33 (2C), 102.22, 63.06, 28.89, 14.53, 6.96, 6.81; MS (ESI+) m/z 356 $(M + H)^+$; HR-MS: m/z Calcd for $C_{22}H_{21}N_5$: 355.1797. Found: 355.1790.

4-((4-((Cyclopropylamino)(4-fluorophenyl)methyl)-5-methylpyrimidin-2-yl)amino)benzonitrile (1y) Yield: 33%; mp 209.8–212.0 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.09 (s, 1H, NH), 8.32 (s, 1H, pyrimidine H_6), 7.90 (d, J = 8.8 Hz, 2H, Ar$H_{3,5}$), 7.68 (d, J = 8.7 Hz, 2H, Ar$H_{2,6}$), 7.44 (dd, J = 8.4, 5.7 Hz, 2H, Ar′$H_{2,6}$), 7.17 (t, J = 8.8 Hz, 2H, Ar′$H_{3,5}$), 5.06 (d, J = 8.9 Hz, 1H, CH), 2.18 (s, 3H, CH_3), 1.96–1.87 (m, 1H, CH), 0.39–0.30 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 168.96, 161.74 (d, J_{CF} = 243.1 Hz), 159.33, 158.34, 145.63, 137.69, 133.44 (2C), 130.24 (2C, d, J_{CF} = 8.0 Hz), 120.53, 120.16, 118.35 (2C), 115.50 (2C, d, J = 21.3 Hz), 102.25, 62.34, 28.90, 14.50, 6.99, 6.78; MS (ESI+) m/z 374 $(M + H)^+$; HR-MS: m/z Calcd for $C_{22}H_{20}FN_5$: 373.1703. Found: 373.1718.

4-((4-((4-Chlorophenyl)(cyclopropylamino)methyl)-5-methylpyrimidin-2-yl)amino)benzonitrile (1z) Yield: 33%; mp 215.6–216.4 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.08 (s, 1H, NH), 8.32 (s, 1H, pyrimidine H_6), 7.86 (d, J = 8.6 Hz, 2H, Ar$H_{3,5}$), 7.67 (d, J = 8.6 Hz, 2H, Ar$H_{2,6}$), 7.42 (s, 4H, Ar′H), 5.06 (d, J = 10.2 Hz, 1H, CH), 2.19 (s, 3H, CH_3), 2.01–1.85 (m 1H, CH), 0.40–0.26 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 168.71, 159.40, 158.30, 145.60, 140.60, 133.42 (2C), 132.11, 130.24 (2C), 128.72 (2C), 120.63, 120.17, 118.34 (2C), 102.24, 62.48, 28.94, 14.51, 7.06, 6.76; 390.59; MS (ESI+) m/z 390 $(M + H)^+$; HR-MS: m/z Calcd for $C_{22}H_{20}ClN_5$: 389.1407. Found: 389.1415.

4-((4-((4-Bromophenyl)(cyclopropylamino)methyl)-5-methylpyrimidin-2-yl)amino)benzonitrile (1aa) Yield: 40%; mp

235.7–237.2 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.07 (s, 1H, NH), 8.32 (s, 1H, pyrimidine H_6), 7.85 (d, J = 8.6 Hz, 2H, Ar$H_{3,5}$), 7.66 (d, J = 8.5 Hz, 2H, Ar$H_{2,6}$), 7.55 (d, J = 8.1 Hz, 2H, Ar′$H_{2,6}$), 7.37 (d, J = 8.1 Hz, 2H, Ar′$H_{3,5}$), 5.05 (d, J = 9.1 Hz, 1H, CH), 2.19 (s, 3H, CH_3), 1.99–1.88 (m, 1H, CH), 0.40–0.28 (m, 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 168.65, 159.40, 158.29, 145.59, 141.04, 133.41 (2C), 131.63 (2C), 130.62 (2C), 120.66, 120.16, 118.34 (2C), 110.01, 102.24, 62.57, 28.95, 14.51, 7.07, 6.75; MS (ESI+) m/z 434 $(M+H)^+$; HR-MS: m/z Calcd for $C_{22}H_{20}BrN_5$: 433.0902. Found: 433.0916.

4-((4-((4-(*tert*-Butyl)phenyl)(cyclopropylamino)methyl)-5-methylpyrimidin-2-yl)amino)benzonitrile (1ab) Yield: 23%; mp 204.4–205.4 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.10 (s, 1H, NH), 8.30 (s, 1H, pyrimidine H_6), 7.95 (d, J = 8.8 Hz, 2H, Ar$H_{3,5}$), 7.70 (d, J = 8.7 Hz, 2H, Ar$H_{2,6}$), 7.33 (q, J = 8.4 Hz, 4H, Ar′H), 4.99 (d, J = 10.4 Hz, 1H, CH), 2.17 (s, 3H, CH_3), 1.95–1.87 (m 1H, CH), 1.24 (s, 9H, *t*-Bu), 0.40–0.28 (m 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 169.33, 159.14, 158.37, 149.88, 145.69, 138.39, 133.50 (2C), 127.88 (2C), 125.58 (2C), 120.38, 120.17, 118.34 (2C), 102.21, 62.61, 34.65, 31.60, 28.85, 14.55, 6.88; MS (ESI+) m/z 412 $(M+H)^+$; HR-MS: m/z Calcd for $C_{26}H_{29}N_5$: 411.2423. Found: 411.2431.

4-((4-(((Cyclopropylamino)(4-methoxyphenyl)methyl)-5-methylpyrimidin-2-yl)amino)benzonitrile (1ac) Yield: 21%; mp 210.9–212.7 °C; ^{1}H NMR (400 MHz, DMSO) δ 10.09 (s, 1H, NH), 8.29 (s, 1H, pyrimidine H_6), 7.94 (d, J = 8.8 Hz, 2H, Ar$H_{3,5}$), 7.70 (d, J = 8.7 Hz, 2H, Ar$H_{2,6}$), 7.29 (d, J = 8.6 Hz, 2H, Ar′$H_{2,6}$), 6.89 (d, J = 8.6 Hz, 2H, Ar′$H_{3,5}$), 4.97 (s, 1H, CH), 3.71 (s, 3H, OCH_3), 2.14 (s, 3H, CH_3), 1.95–1.87 (m, 1H, CH), 0.38–0.27 (m 4H, C_2H_4); ^{13}C NMR (100 MHz, DMSO) δ 169.41, 159.08, 158.79, 158.31, 145.69, 145.08, 133.49 (2C), 133.33, 129.39 (2C), 120.35, 118.33 (2C), 114.20 (2C), 102.20, 62.37, 55.50, 28.77, 14.49, 6.91; MS (ESI+) m/z 386 $(M+H)^+$; HR-MS: m/z Calcd for $C_{23}H_{23}N_5O$: 385.1903. Found: 385.1913.

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-diphenyl tetrazolium bromide (MTT) method. Briefly, virus stocks were titrated in MT-4 cells and expressed as the 50% cell culture infective dose ($CCID_{50}$). MT-4 cells were suspended in culture medium at 1×10^5 cells/mL and infected with HIV at a multiplicity of infection of 0.02. Immediately after 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. 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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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejps.2014.06.003.

References

Antunes, A.M., Novais, D.A., da Silva, J.F., Santos, P.P., Oliveira, M.C., Beland, F.A., Marques, M.M., 2011. Synthesis and oxidation of 2-hydroxynevirapine, a metabolite of the HIV reverse transcriptase inhibitor nevirapine. Org. Biomol. Chem. 9, 7822–7835.

Chen, X., Zhan, P., Li, D., De Clercq, E., Liu, X., 2011. Recent advances in DAPYs and related analogues as HIV-1 NNRTIs. Curr. Med. Chem. 18, 359–376.

Das, K., Bauman, J.D., Clark, A.D., Frenkel, Y.V., Lewi, P.J., Shatkin, A.J., Hughes, S.H., Arnold, E., 2008. High-resolution structures of HIV-1 reverse transcriptase/TMC278 complexes: strategic flexibility explains potency against resistance mutations. Proc. Natl. Acad. Sci. 105, 1466–1471.

de Béthune, M.-P., 2010. Non-nucleoside reverse transcriptase inhibitors (NNRTIs), their discovery, development, and use in the treatment of HIV-1 infection: a review of the last 20 years (1989–2009). Antiviral Res. 85, 75–90.

Feng, X.-Q., Liang, Y.-H., Zeng, Z.-S., Chen, F.-E., Balzarini, J., Pannecouque, C., De Clercq, E., 2009. Structural modifications of DAPY analogues with potent anti-HIV-1 activity. ChemMedChem 4, 219–224.

Feng, X.-Q., Zeng, Z.-S., Liang, Y.-H., Chen, F.-E., Pannecouque, C., Balzarini, J., De Clercq, E., 2010. Synthesis and biological evaluation of 4-(hydroxyimino) arylmethyl diarylpyrimidine analogues as potential non-nucleoside reverse transcriptase inhibitors against HIV. Bioorg. Med. Chem. 18, 2370–2374.

Gu, S.-X., He, Q.-Q., Yang, S.-Q., Ma, X.-D., Chen, F.-E., De Clercq, E., Balzarini, J., Pannecouque, C., 2011. Synthesis and structure–activity relationship of novel diarylpyrimidines with hydromethyl linker (CH(OH)-DAPYs) as HIV-1 NNRTIs. Bioorg. Med. Chem. 19, 5117–5124.

Gu, S.-X., Li, Z.-M., Ma, X.-D., Yang, S.-Q., He, Q.-Q., Chen, F.-E., De Clercq, E., Balzarini, J., Pannecouque, C., 2012. Chiral resolution, absolute configuration assignment and biological activity of racemic diarylpyrimidine CH(OH)-DAPY as potent nonnucleoside HIV-1 reverse transcriptase inhibitors. Eur. J. Med. Chem. 53, 229–234.

Hassam, M., Basson, A.E., Liotta, D.C., Morris, L., van Otterlo, W.A., Pelly, S.C., 2012. Novel cyclopropyl-indole derivatives as HIV non-nucleoside reverse transcriptase inhibitors. ACS Med. Chem. Lett. 3, 470–475.

Jain, A.N., 2009. Effects of protein conformation in docking: improved pose prediction through protein pocket adaptation. J. Comput. Aided Mol. Des. 23, 355–374.

Ma, X.-D., Yang, S.-Q., Gu, S.-X., He, Q.-Q., Chen, F.-E., De Clercq, E., Balzarini, J., Pannecouque, C., 2011. Synthesis and anti-HIV activity of aryl-2-[(4-cyanophenyl) amino]-4-pyrimidinone hydrazones as potent non-nucleoside reverse transcriptase inhibitors. ChemMedChem 6, 2225–2232.

Meng, G., Chen, F., De Clercq, E., Balzarini, J., Pannecouque, C., 2003. Nonnucleoside HIV-1 reverse transcriptase inhibitors: Part I. Synthesis and structure–activity relationship of 1-alkoxymethyl-5-alkyl-6-naphthylmethyl uracils as HEPT analogues. Chem. Pharm. Bull. (Tokyo) 51, 779–789.

Meng, G., Kuang, Y., Ji, L., Chen, F.e., 2005. Synthesis of 1-[(2-hydroxyethoxy)methyl]-6-(5,6,7,8-tetrahydronaphthylmethyl-1)thymine as novel inhibitor against drug-resistant HIV mutants. Synth. Commun. 35, 1095–1102.

Mordant, C., Schmitt, B., Pasquier, E., Demestre, C., Queguiner, L., Masungi, C., Peeters, A., Smeulders, L., Bettens, E., Hertogs, K., 2007. Synthesis of novel diarylpyrimidine analogues of TMC278 and their antiviral activity against HIV-1 wild-type and mutant strains. Eur. J. Med. Chem. 42, 567–579.

Spitzer, R., Jain, A.N., 2012. Surflex-Dock: docking benchmarks and real-world application. J. Comput. Aided Mol. Des. 26, 687–699.

Thakur, A., Thakur, M., Bharadwaj, A., Thakur, S., 2008. SAR and QSAR studies: modelling of new DAPY derivatives. Eur. J. Med. Chem. 43, 471–477.

Tian, X., Qin, B., Wu, Z., Wang, X., Lu, H., Morris-Natschke, S.L., Chen, C.H., Jiang, S., Lee, K.-H., Xie, L., 2010. Design, synthesis, and evaluation of diarylpyridines and diarylanilines as potent non-nucleoside HIV-1 reverse transcriptase inhibitors. J. Med. Chem. 53, 8287–8297.

Zeng, Z.-S., Liang, Y.-H., Feng, X.-Q., Chen, F.-E., Pannecouque, C., Balzarini, J., De Clercq, E., 2010. Lead optimization of diarylpyrimidines as non-nucleoside Inhibitors of HIV-1 reverse transcriptase. ChemMedChem 5, 837–840.

Zhan, P., Liu, X., Li, Z., 2009. Recent advances in the discovery and development of novel HIV-1 NNRTI platforms: 2006–2008 update. Curr. Med. Chem. 16, 2876–2889.