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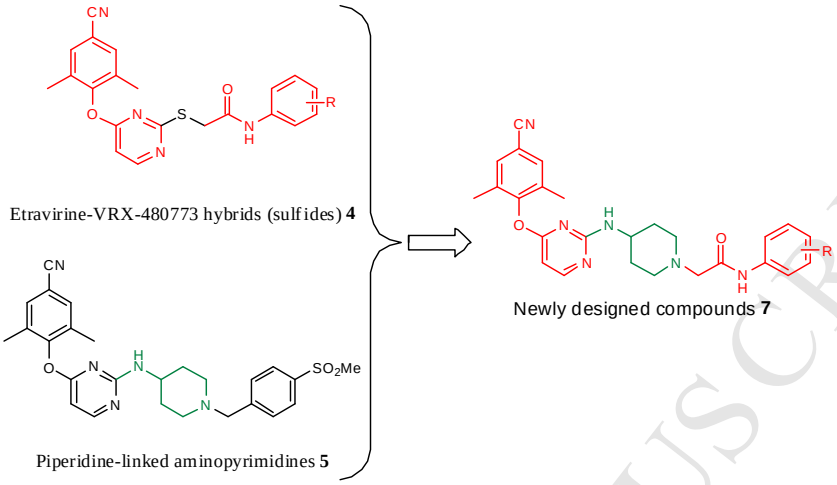

Graphical abstract

Discovery of Piperidin-4-yl-aminopyrimidine Derivatives as Potent Non-nucleoside HIV-1 Reverse Transcriptase Inhibitors

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Abstract:

A novel series of piperidin-4-yl-aminopyrimidine derivatives were designed fusing the pharmacophore templates of etravirine–VRX-480773 hybrids our group previously described and piperidine-linked aminopyrimidines. Most compounds displayed significantly improved activity against wild-type HIV-1 with EC_{50} values in single-digit nanomolar concentrations compared to etravirine–VRX-480773 hybrids. Selected compounds were also evaluated for activity against reverse transcriptase, and had lower IC_{50} values than that of nevirapine. The improved potency observed in this *in vitro* model of HIV RNA replication partly validates the mechanism by which this class of allosteric pyrimidine derivatives inhibits reverse transcriptase, and represents a remarkable step forward in the development of AIDS therapeutics.

Keywords: *Antiviral agent; Biological activity; DAPY; NNRTI; Piperidine*

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1. Introduction

Reverse transcriptase (RT) is one of the most important enzymes in the HIV-1 life cycle. There are two known drug-target sites, the substrate binding site and an allosteric site. The allosteric site is distinct from, but closely located to, the substrate binding site [1]. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) interact with the allosteric binding site on HIV-1 RT in a noncompetitive manner, and disrupt the molecular conformation required for RT catalytic function [2]. Diarylpyrimidine (DAPY) analogues represent a class of highly potent NNRTIs with micromolar to nanomolar activity against wild-type (WT) human immunodeficiency virus type 1 (HIV-1) and clinically relevant mutant HIV-1 strains [3-6]. In the DAPY series, etravirine (ETV, **1**, Figure 1) and rilpivirine (RPV, **2**, Figure 1) were approved by the US Food and Drug Administration (FDA) in January 2008 and May 2011, respectively [7,8]. In our previous studies, we combined the pharmacophoric group of etravirine **1** and VRX-480773 **3** [9] using structure-guided molecular hybridization strategy, which led to the identification of etravirine–VRX-480773 hybrids **4** (Figure 1) with significant potency against WT HIV-1 strain (IIIB) at micromolar concentrations (EC_{50} = 0.24–41 μM), and selectivity index (SI) values ranging from 1 to > 1225 [10]. Unfortunately, these sulfides lost their activity against the most common double mutant strain of HIV-1 (RES056, K103N + Y181C) [10].

The pharmacokinetic profiles of most DAPYs are not satisfactory due to low water solubility [11,12]. Recently, in order to enhance oral bioavailability, a series of piperidine-linked aminopyrimidine derivatives (**5** and **6**, Figure 1) were reported with

good potency against WT and several important resistant mutant strains of HIV-1 in both cellular and enzymic assays [13,14]. Inspired by good potency and bioavailability profiles, we designed a new piperidin-4-yl-aminopyrimidine derivatives **7** by replacing the sulfide functionality in etravirine–VRX-480773 hybrids **4** with a piperidin-4-yl-amino group (Figure 2). A direct molecular modeling comparison (Figure 3) suggested that the newly designed compound **7a** makes bidentate hydrogen bond interactions with the backbone carbonyl and α–amino of residue Lys101, similar to that observed in etravirine, while only one hydrogen bond was developed between the original lead compound sulfide **4a** and residue Lys101. It is worth noting that the amide amino of compound **7a** formed a critical hydrogen bond interaction with the backbone carbonyl of the highly conserved residue Leu234. This could generate new variants of piperidine-linked aminopyrimidine displaying improved activity against WT virus and should have improved activities against mutant strains of HIV-1. Herein, we report their synthesis, anti-HIV evaluation and preliminary structure-activity relationships (SAR) of these piperidin-4-yl-aminopyrimidine derivatives.

Insert Figure 1 here

Insert Figure 2 here

Insert Figure 3 here

2. Results and discussion

2.1. Chemistry

The synthetic route to the target compounds **7a-z** is outlined in Scheme 1. The key

intermediate, ether **8**, was prepared in two steps from pyrimidine-2,4(1*H*, 3*H*)-dione according to previously reported method [10,13,14]. Treatment of ether **8** with 4-amino-1-Boc-piperidine afforded *tert*-butyl 4-((4-(4-cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidine-1-carboxylate (**9**) [13]. By removing the Boc group of compound **9** in the presence of trifluoroacetic acid (TFA) at room temperature, the secondary amine **10** was synthesized with a good yield [13]. Compound **10** was then reacted with substituted α-bromoacetamide, achieving the target compounds **7a-z**.

Insert Scheme 1 here

2.2. Biological activity

The newly synthesized piperidin-4-yl-aminopyrimidine derivatives were evaluated for their anti-HIV activity in MT-4 cell cultures infected with either a WT HIV-1 strain (IIIB), a double mutant HIV-1 strain RES056 (K103N + Y181C) or an HIV-2 strain (ROD). The results were expressed as cytotoxicity (CC_{50}), anti-HIV activity (EC_{50}) and selectivity index (SI = CC_{50}/EC_{50}). The selectivity index indicates the specificity of the antiviral effect. Results are illustrated in Table 1, together with those of the three FDA approved drugs: delavirdine (DEV), efavirenz (EFV) and etravirine (ETV) as the reference standards.

As listed in Table 1, compounds **7a-z** showed moderate to excellent inhibitory activity against WT HIV-1 with EC_{50} values ranging from 304 to 1.9 nM, superior to

that of reference compound DEV (EC_{50} = 657 nM). Eighteen out of 26 newly synthesized compounds proved to be highly effective in inhibiting HIV-1 replication at single-digit nanomolar concentration. These were comparable to, or more active than, the reference drugs EFV and ETV. Additionally, piperidin-4-yl-aminopyrimidine **7a** (EC_{50} = 3.3 nM, SI = 551) was significantly more active than the corresponding lead compound sulfide **4a** (EC_{50} > 320 nM, SI = 1) [10]. The antiviral activity further validated our design. However, compounds **7** lack potency against the double mutant strain RES056 and HIV-2.

The preliminary SARs analysis indicated that the substitution pattern on the right wing of piperidin-4-yl-aminopyrimidine derivatives **7** might play a determinant role in their anti-HIV-1 (ⅢB) activity. In the case of di- or tri- over mono-substitution of the phenyl ring, 2-methyl-4-sulfamoyl-substituted **7z** (EC_{50} = 5.3 nM) showed no marked improvement in its ability to inhibit WT virus compared to the 2-methyl **7b** and 4-sulfamoyl **7y** analogs (EC_{50} = 61.6 and 1.9 nM, respectively). Interestingly, the inhibitory potency was higher for the *para*-substituted derivatives (**7d**, **7j**, **7m** and **7w**) than for the *meta*-substituted analogues (**7c**, **7i**, **7l** and **7v**). The *meta*-substituted analogues were in turn more active than the *ortho*-substituted congeners (**7b**, **7h**, **7k** and **7u**). As was expected, for the compounds with a mono-substituent at the C-4 position of the phenyl ring that was adjacent to the enzyme/water interface [12,15] (Figure 3), the hydrophilic groups (**7x** and **7y**) seem to be more advantageous than the hydrophobic substituents (**7d**, **7g**, **7j**, **7m**, **7r**, and **7w**). This was except for the fact that the introduction of a cyano group at the *para* position of **7a** created compound **7s**,

for which the inhibitory activity was slightly greater.

Insert Table 1 here

To verify their binding target, eleven selected title compounds were assessed in enzyme assays against highly purified recombinant HIV-1 RT (Table 2). Poly(rA)/oligo(dT)$_{16}$ was used as a template and primer, and NVP and EFV as reference [16,17]. All of the compounds tested exhibited moderate inhibitory activity against WT HIV-1 RT (IC_{50} = 0.102–0.207 μM), which was greater than that for NVP (IC_{50} = 1.795 μM), but lower than that for EFV (IC_{50} = 0.044 μM). These data suggest that these piperidin-4-yl-aminopyrimidine derivatives bind to HIV-1 RT and belong to the group of HIV-1 NNRTIs.

Insert Table 2 here

3. Conclusion

To improve the potency of prototypic etravirine–VRX-480773 hybrids **4**, structure-based design of new inhibitors that interact with the highly conserved residue Leu234 and make bidentate hydrogen bond interactions with residue Lys101 has led to the discovery of novel piperidin-4-yl-aminopyrimidine derivatives as highly potent NNRTIs. Screening results indicated that most compounds showed excellent activity against WT HIV-1 with EC_{50} values in the single-digit nanomolar

concentration range. Taking full use of the information from SARs analysis and molecular modeling calculations, further optimization of piperidin-4-yl-aminopyrimidine derivatives to improve their drug resistance profiles are ongoing and will be reported in due course.

4. Experimental section

4.1. Chemistry

Chemical reagents and solvents, purchased from commercial sources, were of analytical grade and were used without further purification. All air-sensitive reactions were run under a nitrogen atmosphere. All the reactions were monitored by TLC on pre-coated silica gel G plates at 254 nm under a UV lamp using ethyl acetate/hexane as eluent. Flash column chromatography was performed with silica gel (300–400 mesh) using ethyl acetate/hexane as eluent. Melting points were measured on a SGW X-1 microscopic melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV400 MHz spectrometer in $CDCl_3$. Chemical shifts were reported in δ (ppm) units relative to the internal standard tetramethylsilane (TMS). IR analysis were carried out on a JASCO FT/IR-4200 series. Mass spectra and HRMS were obtained on a Waters Quattro Micromass instrument and Bruker solari X-70 FT-MS instrument, respectively, using electrospray ionization (ESI) techniques. The purities of target compounds were ≥ 95%, measured by HPLC, performed on an Agilent 1200 HPLC system with UV detector and Agilent Eclipse Plus C_{18} column (150 × 4.6 mm, 5 μm), eluting with a mixture of solvents H_2O (A) and CH_3CN (B) from V_A: V_B = 90:10 to 10:90. Peaks were detected at λ 254 nm with a flow rate of

1.0 mL/min.

4.1.1. Preparation of *tert*-butyl 4-((4-(4-cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidine-1-carboxylate (9)

A mixture of 4-((2-chloropyrimidin-4-yl)oxy)-3,5-dimethylbenzonitrile **8** (3.25 g, 12.5 mmol), 4-amino-1-Boc-piperidine (2.50 g, 12.5 mmol) and DIPEA (4.37 mL, 25 mmol) in NMP (70 mL) was heated at 100 ^{o}C in a sealed tube overnight [13]. Cooling to room temperature, the mixture was poured into vigorously stirred ice water (700 mL), filtered the precipitated solid, washed with chilled water, and dried at 50 ^{o}C under vacuum to obtain the yellow crude product in 90% yield for use in the next step without further purification. The pure product was obtained by flash column chromatography for characterization. Yield 78%; white solid, mp 95.8–96.7 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.24-1.28 (m, 3H, piperidine), 1.44 (s, 9H, 3CH_3), 1.85 (s, 2H, piperidine), 2.13 (s, 6H, 2CH_3), 2.76-3.94 (m, 4H, piperidine), 4.92 (br s, 1H, N*H*), 6.12 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.39 (s, 2H, Ph), 8.13 (d, *J* = 5.6 Hz, 1H, pyrimidine); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.51, 28.53, 31.94, 42.47, 48.56, 79.76, 109.39 (2C), 118.76, 132.40, 133.04, 153.53, 154.81, 160.01, 161.80, 168.62; MS (ESI+) *m/z* 446 $(M+Na)^+$.

4.1.2. Preparation of 3,5-dimethyl-4-((2-(piperidin-4-ylamino)pyrimidin-4-yl)oxy)benzonitrile (10)

Trifluoroacetic acid (12.2 mL, 164.4 mmoL) was added to a solution of **9** (3.48 g,

8.22 mmoL) in DCM (120 mL) at room temperature and stirred overnight [13]. The reaction mixture was neutralized with 2M NaOH to pH = 8. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure and dried at 50 ^oC under vacuum to give the secondary amine **10** as white solid (Yield: 95%) for use in the next step without further purification. The pure product was obtained by flash column chromatography for characterization. Yield 70%; yellow solid, mp 177.7–178.5 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ = 1.46-1.89 (m, 5H, piperidine), 2.13 (s, 6H, 2C*H*$_3$), 2.29-2.99 (m, 4H, piperidine), 3.56 (br s, 1H, N*H*), 5.05 (br s, 1H, N*H*), 6.09 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.38 (s, 2H, Ph), 8.12 (d, *J* = 5.6 Hz, 1H, pyrimidine); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.49, 33.37, 45.37, 48.85, 109.27 (2C), 118.82, 132.34, 133.07, 153.63, 160.05, 161.85, 168.55; MS (ESI+) *m/z* 324 $(M+H)^+$.

4.1.3. General procedure for the preparation of 7a-z

K_2CO_3 (0.2350 g, 1.7 mmoL) was added to a solution of the secondary amine **10** (0.2749 g, 0.85 mmoL) in 2.5 mL of anhydrous DMF at 0 ^oC, followed by stirring for 30 min before 0.85 mmoL of known substituted α-bromoacetamide was added dropwise as a solution in 1.0 mL of anhydrous DMF. The resulting mixture was allowed to stir at 0 ^oC for 2 h and then another 9 h at room temperature. The reaction was monitored by TLC until its completion. The reaction mixture was poured into brine (35 mL), filtered the precipitated solid, washed with chilled water, and dried at 50 ^oC under vacuum to give the corresponding crude product, which was purified by flash column chromatography to afford target compounds **7a-z** in 40-68% yields.

4.1.3.1. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-phenylacetamide (7a)

Yield 51%; white solid, mp 202.7–203.5 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.25-2.04 (m, 5H, piperidine), 2.14 (s, 6H, 2CH_3), 2.34-2.82 (m, 4H, piperidine), 3.11 (s, 2H, CH_2), 4.98 (br s, 1H, N*H*), 6.14 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.10-7.32 (m, 3H, Ph'), 7.40 (s, 2H, Ph), 7.54 (d, *J* = 5.6 Hz, 2H, Ph'), 8.16 (d, *J* = 5.6 Hz, 1H, pyrimidine), 9.08 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.51, 32.32, 47.59, 52.68, 62.15, 109.40 (2C), 118.79, 119.51, 124.32, 129.16, 132.42, 133.04, 137.70, 153.53, 160.07, 161.86, 168.49 (2C); IR (KBr): $\tilde{\nu}$ = 3343 (s; *v*(N-H)), 3234 (s; *v*(N-H)), 2223 (s; *v*(C≡N)), 1691 (s; *v*(C=O)); MS (ESI+) *m/z* 457 $(M+H)^+$; HRMS calcd for $C_{26}H_{28}N_6O_2$ $[M+H]^+$: 457.2352, found: 457.2356; HPLC: t_R = 10.90 min, 98.06%.

4.1.3.2. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(*o*-tolyl)acetamide (7b)

Yield 49%; white solid, mp 207.9–208.6 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.47-2.04 (m, 5H, piperidine), 2.14 (s, 6H, 2CH_3), 2.26 (s, 3H, CH_3), 2.39-2.88 (m, 4H, piperidine), 3.15 (s, 2H, CH_2), 4.98 (br s, 1H, N*H*), 6.14 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.01-7.24 (m, 3H, Ph'), 7.40 (s, 2H, Ph), 8.13-8.17 (m, 2H, pyrimidine + Ph'), 9.24 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.53, 21.48, 32.33, 47.60, 52.66, 62.18, 109.37 (2C), 117.20, 118.81, 126.04, 127.68, 127.71, 132.42, 133.04, 137.52, 138.90, 153.52, 160.12, 161.86, 168.37 (2C); MS (ESI+) *m/z* 471 $(M+H)^+$; HRMS calcd for $C_{27}H_{30}N_6O_2$ $[M+H]^+$: 471.2508, found: 471.2509; HPLC: t_R = 11.33

min, 97.26%.

4.1.3.3. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(*m*-tolyl)acetamide (7c)

Yield 47%; white solid, mp 199.0–199.4 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.48-2.04 (m, 5H, piperidine), 2.14 (s, 6H, 2CH_3), 2.34 (s, 5H, CH_3 + piperidine), 2.81 (d, *J* = 8.4 Hz, 2H, piperidine), 3.10 (s, 2H, CH_2), 4.97 (br s, 1H, N*H*), 6.13 (d, *J* = 5.6 Hz, 1H, pyrimidine), 6.91-7.40 (m, 6H, Ph + Ph'), 8.15 (d, *J* = 5.6 Hz, 1H, pyrimidine), 9.02 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.53, 21.60, 32.33, 47.55, 52.70, 62.15, 109.36 (2C), 116.57, 118.81, 120.08, 125.11, 128.99, 132.42, 133.03, 137.58, 139.10, 153.51, 160.10, 161.86, 168.44 (2C); MS (ESI+) *m/z* 471 $(M+H)^+$; HRMS calcd for $C_{27}H_{30}N_6O_2$ $[M+H]^+$: 471.2508, found: 471.2507; HPLC: t_R = 11.43 min, 98.75%.

4.1.3.4. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(*p*-tolyl)acetamide (7d)

Yield 55%; white solid, mp 185.5–185.9 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.47-2.03 (m, 5H, piperidine), 2.14 (s, 6H, 2CH_3), 2.30 (s, 5H, CH_3 + piperidine), 2.80 (d, *J* = 8.8 Hz, 2H, piperidine), 3.09 (s, 2H, CH_2), 4.97 (br s, 1H, N*H*), 6.13 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.11-7.44 (m, 6H, Ph + Ph'), 8.15 (d, *J* = 5.6 Hz, 1H, pyrimidine), 9.00 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.52, 20.98, 32.32, 47.58, 52.66, 62.12, 109.36 (2C), 118.81, 119.51, 129.62, 132.41, 133.03, 133.90, 135.14, 153.51, 160.10, 161.85, 168.33 (2C); MS (ESI+) *m/z* 471 $(M+H)^+$; HRMS calcd for $C_{27}H_{30}N_6O_2$ $[M+H]^+$: 471.2508, found: 471.2509; HPLC: t_R = 11.29 min,

96.14%.

4.1.3.5. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(3,5-dimethylphenyl)acetamide (7e)

Yield 57%; white solid, mp 205.0–205.4 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.48-1.98 (m, 5H, piperidine), 2.14 (s, 6H, 2CH_3), 2.29 (s, 8H, 2CH_3 + piperidine), 2.80 (d, J = 10.8 Hz, 2H, piperidine), 3.09 (s, 2H, CH_2), 4.96 (br s, 1H, N*H*), 6.13 (d, J = 5.6 Hz, 1H, pyrimidine), 6.75 (s, 1H, Ph'), 7.18 (s, 2H, Ph'), 7.40 (s, 2H, Ph), 8.15 (d, J = 5.6 Hz, 1H, pyrimidine), 8.97 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.54, 17.82, 32.58, 47.57, 52.79, 62.32, 109.36 (2C), 118.80, 120.88, 126.71, 130.43, 132.42, 133.03, 135.90, 153.49, 160.08, 161.85, 168.23 (2C); MS (ESI+) *m/z* 485 $(M+H)^+$; HRMS calcd for $C_{28}H_{32}N_6O_2$ $[M+H]^+$: 485.2665, found: 485.2660; HPLC: t_R = 12.18 min, 97.86%.

4.1.3.6. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(3,4-dimethylphenyl)acetamide (7f)

Yield 62%; white solid, mp 225.6–226.4 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.48-1.98 (m, 5H, piperidine), 2.14 (s, 6H, 2CH_3), 2.21 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 2.30-2.83 (m, 4H, piperidine), 3.09 (s, 2H, CH_2), 4.96 (br s, 1H, N*H*), 6.13 (d, J = 5.6 Hz, 1H, pyrimidine), 7.06-7.31 (m, 3H, Ph'), 7.40 (s, 2H, Ph), 8.15 (d, J = 5.6 Hz, 1H, pyrimidine), 8.95 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.52, 19.30, 19.99, 32.33, 47.58, 52.66, 62.13, 109.35 (2C), 116.98, 118.81, 120.79, 130.10, 132.41, 132.62, 133.02, 135.40, 137.39, 153.52, 160.09, 161.85, 168.29 (2C); MS (ESI+) *m/z* 485 $(M+H)^+$; HRMS calcd for $C_{28}H_{32}N_6O_2$ $[M+H]^+$: 485.2665, found:

485.2669; HPLC: t_R = 12.21 min, 97.80%.

4.1.3.7. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(4-methoxyphenyl)acetamide (7g)

Yield 45%; white solid, mp 194.2–194.9 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ = 1.47-2.03 (m, 5H, piperidine), 2.14 (s, 6H, 2CH_3), 2.30-2.83 (m, 4H, piperidine), 3.09 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3), 4.98 (br s, 1H, N*H*), 6.13 (d, *J* = 5.6 Hz, 1H, pyrimidine), 6.85 (d, *J* = 8.8 Hz, 2H, Ph'), 7.39 (s, 2H, Ph), 7.44 (d, *J* = 8.8 Hz, 2H, Ph'), 8.15 (d, *J* = 5.6 Hz, 1H, pyrimidine), 8.95 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.51, 32.30, 47.61, 52.71, 55.60, 62.04, 109.36 (2C), 114.28, 118.80, 121.19, 130.90, 132.41, 133.03, 153.51, 156.40, 160.09, 161.86, 168.21 (2C); MS (ESI+) *m/z* 487 $(M+H)^+$; HRMS calcd for $C_{27}H_{30}N_6O_3$ $[M+H]^+$: 487.2458, found: 487.2462; HPLC: t_R = 10.82 min, 98.23%.

4.1.3.8. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(2-fluorophenyl)acetamide (7h)

Yield 68%; white solid, mp 224.7–225.5 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ = 1.49-2.03 (m, 5H, piperidine), 2.14 (s, 6H, 2CH_3), 2.30-2.85 (m, 4H, piperidine), 3.14 (s, 2H, CH_2), 5.05 (br s, 1H, N*H*), 6.13 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.00-7.14 (m, 3H, Ph'), 7.39 (s, 2H, Ph), 8.15 (d, *J* = 5.6 Hz, 1H, pyrimidine), 8.34 (t, *J* = 8.0 Hz, 1H, Ph'), 9.53 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.54, 32.42, 47.64, 52.74, 62.11, 109.35 (2C), 114.97 (d, $J_{C\text{-}C\text{-}F}$ = 18.8 Hz), 118.82, 121.24 (d, $J_{C\text{-}C\text{-}F}$ = 22.4 Hz), 124.29 (d, $J_{C3\text{-}F}$ = 7.6 Hz), 124.75 (d, $J_{C4\text{-}F}$ = 3.6 Hz), 126.33 (d, $J_{C3\text{-}F}$ = 10.0 Hz), 132.42, 133.03, 151.28 (d, $J_{C\text{-}F}$ = 225.2 Hz), 153.70, 160.07, 161.86, 168.70 (2C);

MS (ESI+) *m/z* 475 $(M+H)^+$; HRMS calcd for $C_{26}H_{27}FN_6O_2$ $[M+H]^+$: 475.2258, found: 475.2261; HPLC: t_R = 11.43 min, 98.55%.

4.1.3.9. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(3-fluorophenyl)acetamide (7i)

Yield 45%; white solid, mp 214.9–215.7 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ = 1.51-2.07 (m, 5H, piperidine), 2.17 (s, 6H, 2CH_3), 2.33-2.86 (m, 4H, piperidine), 3.14 (s, 2H, CH_2), 5.00 (br s, 1H, N*H*), 6.17 (d, *J* = 5.6 Hz, 1H, pyrimidine), 6.80-7.32 (m, 3H, Ph'), 7.43 (s, 2H, Ph), 7.54 (d, *J* = 10.8 Hz, 1H, Ph'), 8.19 (d, *J* = 5.6 Hz, 1H, pyrimidine), 9.18 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.53, 32.32, 47.49, 52.68, 62.05, 107.09 (d, $J_{C\text{-}C\text{-}F}$ = 26.0 Hz), 109.35 (2C), 111.11 (d, $J_{C\text{-}C\text{-}F}$ = 21.2 Hz), 114.71 (d, $J_{C4\text{-}F}$ = 2.8 Hz), 118.82, 130.26 (d, $J_{C3\text{-}F}$ = 9.2 Hz), 132.42, 133.03, 139.21 (d, $J_{C3\text{-}F}$ = 10.8 Hz), 153.51, 160.12, 161.93, 164.36 (d, $J_{C\text{-}F}$ = 254.0 Hz), 168.67 (2C); MS (ESI+) *m/z* 475 $(M+H)^+$; HRMS calcd for $C_{26}H_{27}FN_6O_2$ $[M+H]^+$: 475.2258, found: 475.2252; HPLC: t_R = 11.41 min, 98.46%.

4.1.3.10. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(4-fluorophenyl)acetamide (7j)

Yield 40%; white solid, mp 194.4–195.2 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ = 1.47-2.04 (m, 5H, piperidine), 2.14 (s, 6H, 2CH_3), 2.30-2.82 (m, 4H, piperidine), 3.10 (s, 2H, CH_2), 4.93 (br s, 1H, N*H*), 6.13 (d, *J* = 5.6 Hz, 1H, pyrimidine), 6.99 (t, *J* = 8.8 Hz, 2H, Ph'), 7.40 (s, 2H, Ph), 7.49-7.53 (m, 2H, Ph'), 8.15 (d, *J* = 5.6 Hz, 1H, pyrimidine), 9.06 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.52, 32.28, 47.57, 52.73, 62.01, 109.37 (2C), 115.88 (d, $J_{C\text{-}C\text{-}F}$ = 22.4 Hz), 118.80, 121.23 (d, $J_{C3\text{-}F}$ = 7.8

Hz), 132.41, 133.04, 133.77 (d, $J_{C4\text{-}F}$ = 2.7 Hz), 153.51, 160.13, 160.58 (d, $J_{C\text{-}F}$ = 241.8 Hz), 161.85, 168.46 (2C); MS (ESI+) *m/z* 475 $(M+H)^+$; HRMS calcd for $C_{26}H_{27}FN_6O_2$ $[M+H]^+$: 475.2258, found: 475.2252; HPLC: t_R = 10.85 min, 98.58%.

4.1.3.11. *N*-(2-Chlorophenyl)-2-(4-((4-(4-cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)acetamide (7k)

Yield 53%; white solid, mp 215.9–216.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.54-2.05 (m, 5H, piperidine), 2.16 (s, 6H, 2C*H*$_3$), 2.31-2.88 (m, 4H, piperidine), 3.17 (s, 2H, C*H*$_2$), 4.90 (br s, 1H, N*H*), 6.15 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.02-7.41 (m, 5H, Ph + Ph'), 8.17 (d, *J* = 5.6 Hz, 1H, pyrimidine), 8.46 (d, *J* = 8.0 Hz, 1H, Ph'), 9.96 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.54, 32.50, 47.67, 52.76, 62.27, 109.37 (2C), 118.81, 120.96, 122.71, 124.56, 127.92, 129.15, 132.43, 133.05, 134.64, 153.54, 160.09, 161.89, 168.81 (2C); MS (ESI+) *m/z* 491 $(M+H)^+$; HRMS calcd for $C_{26}H_{27}ClN_6O_2$ $[M+H]^+$: 491.1962, found: 491.1968; HPLC: t_R = 12.14 min, 97.43%.

4.1.3.12. *N*-(3-Chlorophenyl)-2-(4-((4-(4-cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)acetamide (7l)

Yield 54%; white solid, mp 206.1–206.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.52-2.08 (m, 5H, piperidine), 2.18 (s, 6H, 2C*H*$_3$), 2.34-2.86 (m, 4H, piperidine), 3.15 (s, 2H, C*H*$_2$), 4.98 (br s, 1H, N*H*), 6.18 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.10-7.67 (m, 6H, Ph + Ph'), 8.20 (d, *J* = 5.6 Hz, 1H, pyrimidine), 9.17 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.53, 32.31, 47.54, 52.72, 62.06, 109.38 (2C), 117.46, 118.81, 119.53, 124.33, 130.18, 132.42, 133.04, 134.79, 138.82, 153.52, 160.11, 161.86, 168.65 (2C); MS (ESI+) *m/z* 491 $(M+H)^+$; HRMS calcd for $C_{26}H_{27}ClN_6O_2$ $[M+H]^+$:

491.1962, found: 491.1959; HPLC: t_R = 11.95 min, 97.44%.

4.1.3.13. *N*-(4-Chlorophenyl)-2-(4-((4-(4-cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)acetamide (7m)

Yield 56%; white solid, mp 204.9–205.5 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ = 1.47-2.04 (m, 5H, piperidine), 2.14 (s, 6H, 2CH_3), 2.30-2.82 (m, 4H, piperidine), 3.10 (s, 2H, CH_2), 4.93 (br s, 1H, N*H*), 6.13 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.27 (d, *J* = 8.8 Hz, 2H, Ph'), 7.39 (s, 2H, Ph), 7.49 (d, *J* = 8.8 Hz, 2H, Ph'), 8.15 (d, *J* = 5.6 Hz, 1H, pyrimidine), 9.10 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.52, 32.30, 47.55, 52.72, 62.04, 109.37 (2C), 118.80, 120.69, 129.14, 129.20, 132.41, 133.04, 136.27, 153.52, 160.13, 161.84, 168.57 (2C); MS (ESI+) *m/z* 491 $(M+H)^+$; HRMS calcd for $C_{26}H_{27}ClN_6O_2$ $[M+H]^+$: 491.1962, found: 491.1957; HPLC: t_R = 11.62 min, 98.85%.

4.1.3.14. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(2,4-dichlorophenyl)acetamide (7n)

Yield 58%; white solid, mp 186.9–187.8 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ = 1.51-2.04 (m, 5H, piperidine), 2.14 (s, 6H, 2CH_3), 2.30-2.86 (m, 4H, piperidine), 3.15 (s, 2H, CH_2), 4.91 (br s, 1H, N*H*), 6.14 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.22-7.40 (m, 4H, Ph + Ph'), 8.15 (d, *J* = 5.6 Hz, 1H, pyrimidine), 8.43 (d, *J* = 8.8 Hz, 1H, Ph'), 9.95 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.54, 32.50, 47.67, 52.76, 62.27, 109.37 (2C), 118.81, 121,04, 127.03, 128.91, 130.63, 131.35, 132.43, 132.76, 133.05, 153.54, 160.09, 161.89, 168.81 (2C); MS (ESI+) *m/z* 525 $(M+H)^+$; HRMS calcd for $C_{26}H_{26}Cl_2N_6O_2$ $[M+H]^+$: 525.1573, found: 525.1567; HPLC: t_R = 13.21 min, 97.46%.

4.1.3.15. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(3,4-dichlorophenyl)acetamide (7o)

Yield 65%; white solid, mp 235.5–236.3 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.47-2.04 (m, 5H, piperidine), 2.14 (s, 6H, 2CH_3), 2.30-2.81 (m, 4H, piperidine), 3.11 (s, 2H, CH_2), 4.94 (br s, 1H, N*H*), 6.14 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.35-7.75 (m, 5H, Ph + Ph'), 8.15 (d, *J* = 5.6 Hz, 1H, pyrimidine), 9.15 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.52, 32.25, 47.54, 52.73, 61.98, 109.37 (2C), 118.73, 118.80, 121,10, 127.39, 130.66, 132.41, 132.90, 133.04, 137.16, 153.52, 160.12, 161.84, 168.71 (2C); MS (ESI+) *m/z* 525 $(M+H)^+$; HRMS calcd for $C_{26}H_{26}Cl_2N_6O_2$ $[M+H]^+$: 525.1573, found: 525.1567; HPLC: t_R = 12.62 min, 98.27%.

4.1.3.16. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(3,5-dichlorophenyl)acetamide (7p)

Yield 49%; white solid, mp 237.5–238.0 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.48-2.04 (m, 5H, piperidine), 2.14 (s, 6H, 2CH_3), 2.30-2.81 (m, 4H, piperidine), 3.11 (s, 2H, CH_2), 4.93 (br s, 1H, N*H*), 6.14 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.08 (s, 1H, Ph'), 7.40 (s, 2H, Ph), 7.51 (s, 2H, Ph'), 8.15 (d, *J* = 5.6 Hz, 1H, pyrimidine), 9.17 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.52, 32.26, 47.52, 52.74, 61.97, 109.37 (2C), 117.68, 118.80, 124.21, 132.41, 133.04, 135.39, 139.45, 153.52, 160.11, 161.85, 168.79 (2C); MS (ESI+) *m/z* 525 $(M+H)^+$; HRMS calcd for $C_{26}H_{26}Cl_2N_6O_2$ $[M+H]^+$: 525.1573, found: 525.1568; HPLC: t_R = 13.08 min, 98.02%.

4.1.3.17. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(2,4,6-trichlorophenyl)acetamide (7q)

Yield 55%; white solid, mp 202.8–203.4 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.50-2.04 (m, 5H, piperidine), 2.14 (s, 6H, 2C*H*$_3$), 2.30-2.96 (m, 4H, piperidine), 3.18 (s, 2H, C*H*$_2$), 4.89 (br s, 1H, N*H*), 6.12 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.39-7.40 (m, 4H, Ph + Ph'), 8.15 (d, *J* = 5.6 Hz, 1H, pyrimidine), 8.93 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.52, 32.28, 47.59, 52.91, 61.69, 109.36 (2C), 118.78, 128.59, 130.96, 132.41, 133.02, 133.33, 133.88, 153.49, 160.08, 161.88, 168.73 (2C); MS (ESI+) *m/z* 559 $(M+H)^+$; HRMS calcd for $C_{26}H_{25}Cl_3N_6O_2$ $[M+H]^+$: 559.1183, found: 559.1187; HPLC: t_R = 11.85 min, 96.83%.

4.1.3.18. *N*-(4-Bromophenyl)-2-(4-((4-(4-cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)acetamide (7r)

Yield 59%; white solid, mp 203.3–204.1 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.47-2.04 (m, 5H, piperidine), 2.14 (s, 6H, 2C*H*$_3$), 2.30-2.82 (m, 4H, piperidine), 3.10 (s, 2H, C*H*$_2$), 4.92 (br s, 1H, N*H*), 6.13 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.39-7.47 (m, 6H, Ph + Ph'), 8.15 (d, *J* = 5.6 Hz, 1H, pyrimidine), 9.10 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.52, 32.28, 47.53, 52.71, 62.05, 109.36 (2C), 116.78, 118.80, 121.02, 132.08, 132.40, 133.03, 136.76, 153.50, 160.12, 161.84, 168.58 (2C); MS (ESI+) *m/z* 535 $(M+H)^+$; HRMS calcd for $C_{26}H_{27}BrN_6O_2$ $[M+H]^+$: 535.1457, found: 535.1459; HPLC: t_R = 11.85 min, 98.16%.

4.1.3.19. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(4-cyanophenyl)acetamide (7s)

Yield 43%; white solid, mp 218.6–219.2 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.47-2.03 (m, 5H, piperidine), 2.14 (s, 6H, 2C*H*$_3$), 2.30-2.82 (m, 4H, piperidine), 3.13

(s, 2H, CH_2), 4.93 (br s, 1H, N*H*), 6.14 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.39 (s, 2H, Ph), 7.59 (d, *J* = 8.4 Hz, 2H, Ph'), 7.67 (d, *J* = 8.4 Hz, 2H, Ph'), 8.15 (d, *J* = 5.6 Hz, 1H, pyrimidine), 9.35 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.51, 32.25, 47.50, 52.69, 62.04, 107.22, 109.37 (2C), 118.79, 118.93, 119.37, 132.39, 133.04, 133.43, 141.59, 153.52, 160.13, 161.82, 169.06 (2C); MS (ESI+) *m/z* 504 $(M+Na)^+$; HRMS calcd for $C_{27}H_{27}N_7O_2$ $[M+H]^+$: 482.2304, found: 482.2309; HPLC: t_R = 10.72 min, 97.64%.

4.1.3.20. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(4-cyano-2-fluorophenyl)acetamide (7t)

Yield 48%; white solid, mp 227.1–227.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.48-2.04 (m, 5H, piperidine), 2.14 (s, 6H, 2CH_3), 2.30-2.81 (m, 4H, piperidine), 3.17 (s, 2H, CH_2), 4.93 (br s, 1H, N*H*), 6.15 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.37-7.46 (m, 4H, Ph + Ph'), 8.15 (d, *J* = 5.6 Hz, 1H, pyrimidine), 8.57 (t, *J* = 8.2 Hz, 1H, Ph'), 9.85 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.52, 32.41, 47.52, 52.74, 62.00, 106.78 (d, $J_{C3\text{-}F}$ = 9.3 Hz), 109.36 (2C), 118.42 (d, $J_{C\text{-}C\text{-}F}$ = 54.1 Hz), 118.65, 118.80, 121.09 (d, $J_{C\text{-}C\text{-}F}$ = 24.5 Hz), 129.71 (d, $J_{C4\text{-}F}$ = 3.5 Hz), 131.08 (d, $J_{C3\text{-}F}$ = 9.8 Hz), 132.41, 133.04, 150.03 (d, $J_{C\text{-}F}$ = 245.1 Hz), 153.54, 160.12, 161.85, 169.24 (2C); MS (ESI+) *m/z* 522 $(M+Na)^+$; HRMS calcd for $C_{27}H_{26}FN_7O_2$ $[M+H]^+$: 500.2210, found: 500.2208; HPLC: t_R = 11.27 min, 97.67%.

4.1.3.21. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(2-nitrophenyl)acetamide (7u)

Yield 48%; white solid, mp 246.9–247.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ =

1.65-2.04 (m, 5H, piperidine), 2.15 (s, 6H, 2CH_3), 2.30-2.86 (m, 4H, piperidine), 3.18 (s, 2H, CH_2), 4.83 (m, 1H, N*H*), 6.14 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.16 (t, *J* = 7.8 Hz, 1H, Ph'), 7.40 (s, 2H, Ph), 7.62 (t, *J* = 7.8 Hz, 1H, Ph'), 8.17 (d, *J* = 5.6 Hz, 1H, pyrimidine), 8.21 (d, *J* = 8.4 Hz, 1H, Ph'), 8.87 (d, *J* = 8.4 Hz, 1H, Ph'),11.94 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.56, 32.15, 47.71, 53.05, 62.56, 109.34 (2C), 118.88, 121.96, 123.32, 125.92, 132.39, 132.44, 132.47, 133.06, 134.62, 153.54, 160.16, 161.87, 170.66 (2C); MS (ESI+) *m/z* 524 $(M+Na)^+$; HRMS calcd for $C_{26}H_{27}N_7O_4$ $[M+H]^+$: 502.2203, found: 502.2198; HPLC: t_R = 11.75 min, 97.88%.

4.1.3.22. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(3-nitrophenyl)acetamide (7v)

Yield 46%; white solid, mp 240.1–240.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.44-1.91 (m, 5H, piperidine), 2.08 (s, 6H, 2CH_3), 2.76-3.12 (m, 4H, piperidine), 3.19 (s, 2H, CH_2), 4.85 (m, 1H, N*H*), 6.21 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.57-8.19 (m, 6H, Ph + Ph'), 8.67 (d, *J* = 5.6 Hz, 1H, pyrimidine), 10.22 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.57, 32.14, 47.75, 53.03, 62.55, 109.36 (2C), 114.54, 118.86, 119.56, 127.74, 129.82, 132.44, 133.06, 139.47, 148.13, 153.54, 160.16, 161.87, 170.64 (2C); MS (ESI+) *m/z* 524 $(M+Na)^+$; HRMS calcd for $C_{26}H_{27}N_7O_4$ $[M+H]^+$: 502.2203, found: 502.2197; HPLC: t_R = 11.49 min, 97.24%.

4.1.3.23. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(4-nitrophenyl)acetamide (7w)

Yield 60%; white solid, mp 234.5–235.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.49-2.04 (m, 5H, piperidine), 2.14 (s, 6H, 2CH_3), 2.30-2.82 (m, 4H, piperidine), 3.16

(s, 2H, CH_2), 4.93 (m, 1H, N*H*), 6.15 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.40 (s, 2H, Ph), 7.72 (d, *J* = 9.2 Hz, 2H, Ph'), 8.16 (d, *J* = 5.6 Hz, 1H, pyrimidine), 8.20 (d, *J* = 9.2 Hz, 2H, Ph'), 9.48 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.52, 32.28, 47.48, 52.77, 62.05, 109.38 (2C), 118.80, 118.93, 125.26, 132.41, 133.05, 143.40, 143.62, 153.52, 160.11, 161.82, 169.16 (2C); MS (ESI+) *m/z* 524 $(M+Na)^+$; HRMS calcd for $C_{26}H_{27}N_7O_4$ $[M+H]^+$: 502.2203, found: 502.2200; HPLC: t_R = 11.13 min, 97.62%.

4.1.3.24. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(4-hydroxyphenyl)acetamide (7x)

Yield 63%; white solid, mp 242.9–243.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.42-2.05 (m, 5H, piperidine), 2.15 (s, 6H, 2CH_3), 2.24-2.80 (m, 4H, piperidine), 3.09 (s, 2H, CH_2), 4.93 (m, 1H, N*H*), 6.14 (d, *J* = 5.6 Hz, 1H, pyrimidine), 6.79-7.40 (m, 6H, Ph + Ph'), 8.16 (d, *J* = 5.6 Hz, 1H, pyrimidine), 8.96 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.53, 32.27, 47.46, 52.75, 62.07, 109.34 (2C), 116.14, 118.83, 123.04, 131.13, 132.40, 133.04, 153.55, 154.15, 160.15, 161.84, 169.17 (2C); MS (ESI+) *m/z* 595 $(M+Na)^+$; HRMS calcd for $C_{26}H_{28}N_6O_3$ $[M+H]^+$: 473.2301, found: 473.2305; HPLC: t_R = 10.25 min, 98.61%.

4.1.3.25. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(4-sulfamoylphenyl)acetamide (7y)

Yield 64%; white solid, mp 228.8–229.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.42-1.92 (m, 5H, piperidine), 2.08 (s, 6H, 2CH_3), 2.76-3.11 (m, 4H, piperidine), 3.06 (s, 2H, CH_2), 4.95 (m, 1H, N*H*), 6.22 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.26 (s, 2H, SO_2NH_2), 7.67 (s, 2H, Ph), 7.74-7.80 (m, 4H, Ph'), 8.19 (d, *J* = 5.6 Hz, 1H,

pyrimidine), 10.04 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.52, 32.28, 47.48, 52.77, 62.05, 109.38 (2C), 188.04, 118.80, 129.43, 132.41, 133.05, 136.52, 141.73, 153.52, 160.11, 161.82, 169.16 (2C); MS (ESI+) *m/z* 558 $(M+Na)^+$; HRMS calcd for $C_{26}H_{29}N_7O_4S$ $[M+H]^+$: 536.2080, found: 536.2079; HPLC: t_R = 9.11 min, 97.65%.

4.1.3.26. 2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(2-methyl-4-sulfamoylphenyl)acetamide (7z)

Yield 66%; white solid, mp 261.3–261.9 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.44-1.98 (m, 5H, piperidine), 2.08 (s, 6H, 2CH_3), 2.31 (s, 3H, CH_3), 2.85-3.12 (m, 4H, piperidine), 3.08 (s, 2H, CH_2), 4.97 (m, 1H, N*H*), 6.24 (d, *J* = 5.6 Hz, 1H, pyrimidine), 7.25 (s, 2H, SO_2NH_2), 7.63-8.12 (m, 5H, Ph + Ph'), 8.20 (d, *J* = 5.6 Hz, 1H, pyrimidine), 9.66 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 15.87, 17.41, 31.57, 47.79, 52.47, 61.55, 108.15 (2C), 111.11, 188.66, 120.11, 124.23, 127.65, 132.19, 132.70, 134.64, 139.05, 153.25, 160.54, 161.63, 168.63 (2C); MS (ESI+) *m/z* 572 $(M+Na)^+$; HRMS calcd for $C_{27}H_{31}N_7O_4S$ $[M+H]^+$: 550.2236, found: 550.2240; HPLC: t_R = 9.61 min, 97.49%.

4.2. Biological evaluation

4.2.1. In vitro anti-HIV assay

The anti-HIV activity and cytotoxicity of the compounds **7a-z** were evaluated against wild-type HIV-1 strain IIIB, a double RT mutant (K103N + Y181C) HIV-1 strain and HIV-2 strain ROD in MT-4 cell cultures using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [18,19]. 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 test compounds were dissolved in DMSO at 50 mM or higher. 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.

4.2.2. HIV-1 RT inhibition assay

Recombinant wild type p66/p51 HIV-1 RT was expressed and purified as previously described [16]. The RT assay was performed with the EnzCheck Reverse Transcriptase Assay kit (Molecular Probes, Invitrogen), as described by the Manufacturer. The assay was based on the dsDNA quantitation reagent PicoGreen. This reagent showed a pronounced increase in fluorescence signal upon binding to dsDNA or RNA-DNA heteroduplexes. Single-stranded nucleic acids generated only minor fluorescence signal enhancement when a sufficiently high dye:base pair ratio was applied [17]. This condition was met in the assay.

A poly(rA) template of approximately 350 bases long, and an oligo$(dT)_{16}$ primer, were annealed in a molar ratio of 1:1.2 (60 min, at room temperature). 52 ng of the RNA/DNA was brought into each well of a 96-well plate in a volume of 20 mL polymerization buffer (60 mM Tris-HCl, 60 mM KCl, 8 mM $MgCl_2$, 13 mM DTT, 100 mM dTTP, pH = 8.1). 5.0 mL of RT enzyme solution, diluted to a suitable

concentration in enzyme dilution buffer (50 mM Tris-HCl, 20% glycerol, 2 mM DTT, pH = 7.6), was added. The reaction mixture was incubated at 25 ^{o}C for 40 min and then stopped by the addition of EDTA (15 mM). Heteroduplexes were then detected by addition of PicoGreen. Signals were read using an excitation wavelength of 490 nm and emission detection at 523 nm using a spectrofluorometer (Safire2, Tecan). To test the activity of compounds against RT, 1.0 mL of compound in DMSO was added to each well before the addition of RT enzyme solution. Control wells without compound contained the same amount of DMSO. Results were expressed as relative fluorescence, i. e. the fluorescence signal of the reaction mixed with compound divided by the signal of the same reaction mixed without compound.

4.3. Molecular docking

Molecular modeling was performed with the Tripos molecular modeling packages Sybyl-X 1.2. All the molecules for docking were built using standard bond lengths and angles from Sybyl-X 1.2/base Builder and were then optimized using the Tripos force field for 2000 generations two times or more, until the minimized conformers of the ligand were the same. The flexible docking method, called Surflex-Dock [20], docks the ligand automatically into the ligand binding site of the receptor by using a protocol-based approach and an empirically-derived scoring function [21]. The protocol is a computational representation of a putative ligand that binds to the intended binding site and is a unique and essential element of the docking algorithm [22]. The scoring function in Surflex-Dock, which contains hydrophobic, polar, repulsive, entropic, and solvation terms, was trained to estimate the dissociation

constant (K_d) expressed in -log $(K_d)^2$. Prior to docking, the protein was prepared by removing water molecules, the ligand **6**, and other unnecessary small molecules from the crystal structure of the **6**-HIV-1 RT complex (PDB code: 3NBP) [14]; simultaneously, polar hydrogen atoms were added to the protein. Surflex-Dock default settings were used for other parameters, such as the number of starting conformations per molecule (set to 0), the size to expand search grid (set to 8 Å), the maximum number of rotatable bonds per molecule (set to 100), and the maximum number of poses per ligand (set to 20). During the docking procedure, all of the single bonds in residue side-chains inside the defined RT binding pocket were regarded as rotatable or flexible, and the ligand was allowed to rotate at all single bonds and move flexibly within the tentative binding pocket. The atomic charges were recalculated using the Kollman all-atom approach for the protein and the Gasteiger-Hückel approach for the ligand. The binding interaction energy was calculated, including van der Waals, electrostatic, and torsional energy terms defined in the Tripos force field. The structure optimization was performed for 20,000 generations using a genetic algorithm, and the 20-best-scoring ligand-protein complexes were kept for further analyses. The -log $(K_d)^2$ values of the 20-best-scoring complexes, which represented the binding affinities of ligand with RT, encompassed a wide scope of functional classes (10^{-2}–10^{-9}). Therefore, only the highest-scoring 3D structural model of the ligand-bound RT was chosen to define the binding interaction [23].

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Figure captions

Figure 1. The structures of etravirine, rilpivirine, VRX-480773, etravirine–VRX-480773 hybrids and piperidine-linked aminopyrimidine derivatives.

Figure 2. The design of piperidin-4-yl-aminopyrimidine derivatives **7**.

Figure 3. Superposition of lower-energy docking binding conformations of sulfide **4a** (white) and piperidin-4-yl-aminopyrimidine **7a** (purple) in the binding pocket of HIV-1 RT.

Table 1

Biological activities of compounds **7a-z** in MT-4 cells[a]

Compd	R	EC_{50}[b] (nM)			CC_{50}[c] (nM)	SI[d]
		III_B	RES056	HIV-2		
4a [10]	H	> 320	> 320	> 320	> 320	1
7a	H	3.3 ± 1.3	> 1818	> 1818	1818 ± 687	551
7b	2-Me	61.6 ± 2.1	> 1254	> 1254	1254 ± 106	20
7c	3-Me	6.6 ± 1.1	> 1679	> 1679	1679 ± 1041	254
7d	4-Me	5.5 ± 1.5	> 2019	> 2019	2019 ± 1254	367
7e	3,5-DiMe	14.9 ± 10.5	> 2311	> 2311	2311 ± 1259	155
7f	3,4-DiMe	8.5 ± 2.3	> 1506	> 1506	1506 ± 1114	177
7g	4-MeO	3.5 ± 2.3	> 1562	> 1562	1562 ± 1110	446
7h	2-F	7.0 ± 0.6	> 118580	> 118580	118580 ± 24319	16940
7i	3-F	6.1 ± 1.5	> 1412	> 1412	1412 ± 695	231
7j	4-F	4.2 ± 2.1	> 1812	> 1812	1812 ± 1222	431
7k	2-Cl	34.6 ± 20.4	> 1507	> 1507	1507 ± 774	44
7l	3-Cl	5.9 ± 1.0	> 1263	> 1263	1263 ± 855	214
7m	4-Cl	6.1 ± 0.8	> 998	> 998	998 ± 570	164
7n	2,4-DiCl	118 ± 53.3	> 1751	> 1751	1751 ± 1142	15
7o	3,4-DiCl	20.9 ± 11.4	> 1275	> 1275	1275 ± 742	61
7p	3,5-DiCl	76.1 ± 26.6	> 3521	> 3521	3521 ± 1351	46
7q	2,4,6-triCl	≥ 304	> 1500	> 1500	1500 ± 679	≤ 5
7r	4-Br	6.2 ± 1.5	> 1475	> 1475	1475 ± 971	238
7s	4-CN	2.9 ± 1.2	> 602	> 602	602 ± 353	208
7t	2-F-4-CN	8.0 ± 1.8	> 1962	> 1962	1962 ± 1441	245
7u	2-NO_2	41.9 ± 16.0	> 59337	> 59337	59337 ± 4923	1416
7v	3-NO_2	7.8 ± 2.6	> 379	> 379	≥ 379	≥ 49
7w	4-NO_2	5.8 ± 3.8	> 518	> 518	518 ± 48	89
7x	4-OH	2.1 ± 0.6	> 2180	> 2180	2180 ± 1524	1038
7y	4-SO_2NH_2	1.9 ± 0.7	> 1606	> 1606	1606 ± 1176	845
7z	2-Me-4-SO_2NH_2	5.3 ± 2.9	> 1637	> 1637	1637 ± 746	309
DEV		657 ± 61.3	> 43806	ND[e]	> 43806	> 67
EFV		6.3 ± 1.6	155 ± 15.8	ND	> 6336	> 1006
ETV		4.1 ± 0.2	25.3 ± 2.3	ND	> 4595	> 1121

[a] All data represent mean values for at least three separate experiments.

[b] EC_{50}: effective concentration required to protect the cell against viral cytopathicity 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} (WT).

[e] ND: not detected.

Table 2

Inhibitory activity of representative piperidin-4-yl-aminopyrimidine against HIV-1 RT[a]

Compd	IC_{50}[b] (μM)
7a	0.199
7d	0.181 ± 0.083
7h	0.207 ± 0.019
7j	0.204 ± 0.025
7t	0.174 ± 0.060
7u	0.193 ± 0.010
7v	0.156 ± 0.022
7w	0.136 ± 0.026
7x	0.102 ± 0.021
7y	0.116 ± 0.009
7z	0.166 ± 0.007
NVP	1.795 ± 0.502
EFV	0.044 ± 0.013

[a] Data represent the mean values of at least two separate experiments.

[b] IC_{50}: inhibitory concentration required to inhibit biotin deoxyuridine triphosphate (biotin-dUTP) incorporation into the HIV-1 RT by 50%.

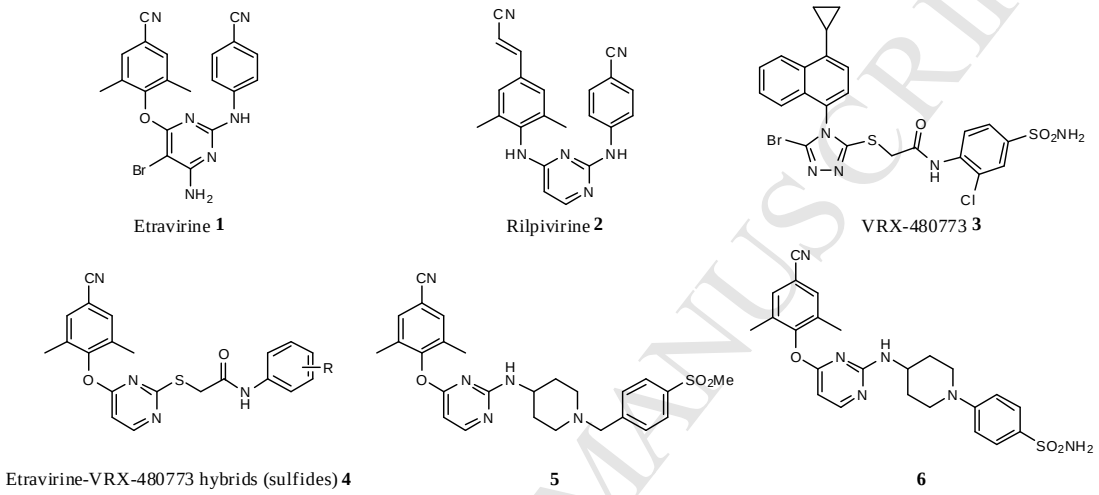


Figure 1. The structures of etravirine, rilpivirine, VRX-480773, etravirine–VRX-480773 hybrids and piperidine-linked aminopyrimidine derivatives.

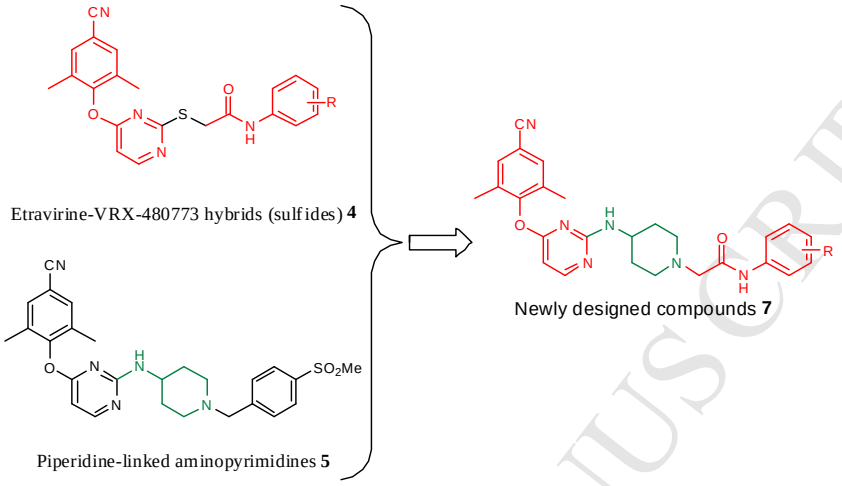


Figure 2. The design of piperidin-4-yl-aminopyrimidine derivatives **7**.

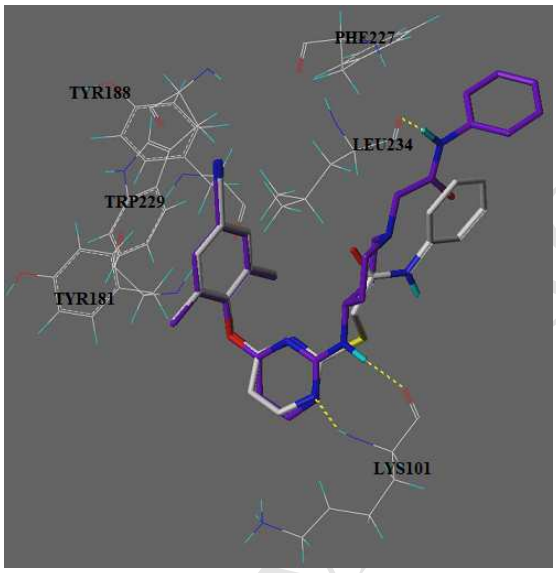
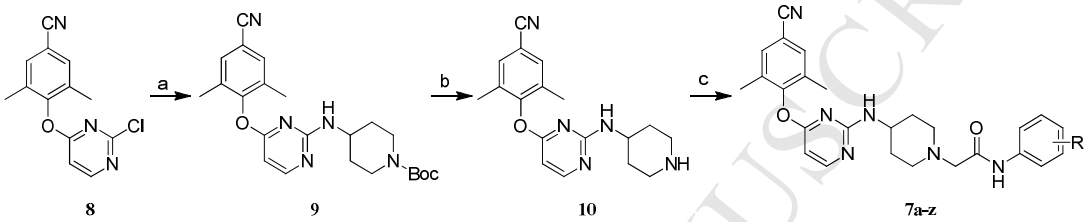


Figure 3. Superposition of lower-energy docking binding conformations of sulfide **4a** (white) and piperidin-4-yl-aminopyrimidine **7a** (purple) in the binding pocket of HIV-1 RT.



Scheme 1. Synthesis of target compounds **7a-z**. Reagents and conditions: (a) 4-amino-1-Boc-piperidine, DIPEA, NMP, 100 ^{o}C, overnight; (b) CF_3COOH, DCM, r.t., overnight; (c) substituted α-bromoacetamide, K_2CO_3, DMF, r.t., overnight.

Discovery of Piperidin-4-yl-aminopyrimidine Derivatives as Potent Non-nucleoside HIV-1 Reverse Transcriptase Inhibitors

Zheng-Yong Wan[a], Jin Yao[a], Yuan Tao[a], Tian-Qi Mao[a,b], Xin-Long Wang[a], Yi-Pei Lu[a], Hai-Feng Wang[a], Hong Yin[a], Yan Wu[a,*], Fen-Er Chen[a,b,*], Erik De Clercq[c], Dirk Daelemans[c], Christophe Pannecouque[c]

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Highlights:

►The etravirine–VRX-480773 hybrids previously disclosed were further optimized.
►Novel piperidin-4-yl-aminopyrimidines were designed using molecular hybridization.
►26 new compounds were designed and synthesized as the anti-drug resistant HIV NNRTIs.
►Most compounds have EC_{50} at single-digit nanomolar concentrations against WT HIV-1.
► SARs were discussed in detail.

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Supporting Information

Discovery of Piperidin-4-yl-aminopyrimidine Derivatives as Potent Non-nucleoside HIV-1 Reverse Transcriptase Inhibitors

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Contents:

^{1}H NMR, ^{13}C NMR and ESI+ of representative compounds

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***tert*-butyl 4-((4-(4-cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidine-1-carboxylate (9)**

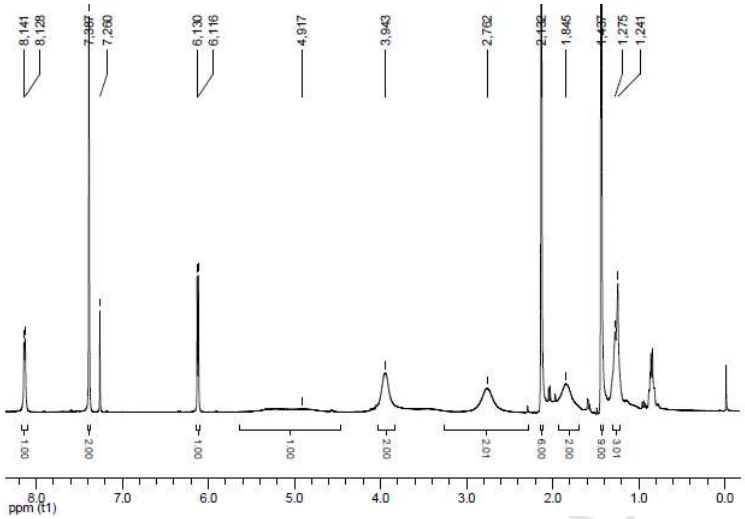

Figure S1: ^{1}H NMR spectrum of compound **9**

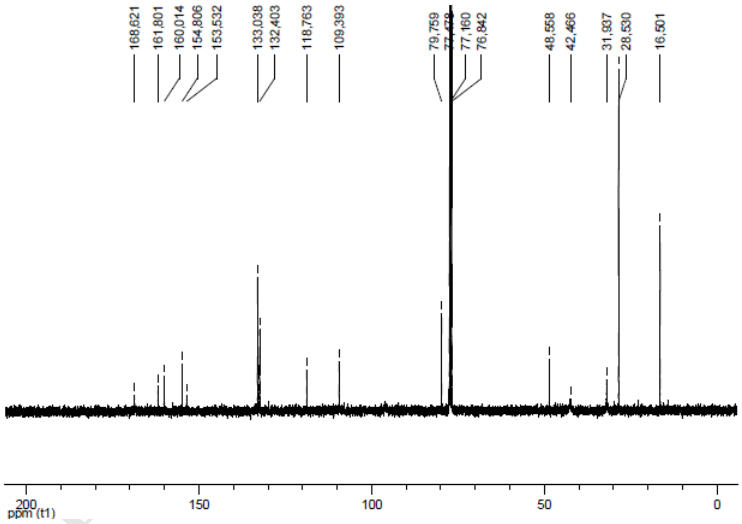

Figure S2: ^{13}C NMR spectrum of compound **9**

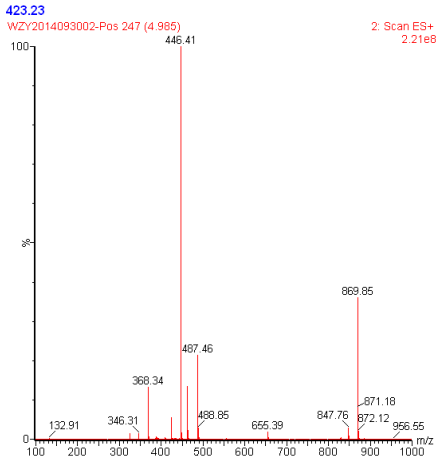

Figure S3: MS spectrum of compound **9**

3,5-dimethyl-4-((2-(piperidin-4-ylamino)pyrimidin-4-yl)oxy)benzonitrile (10)

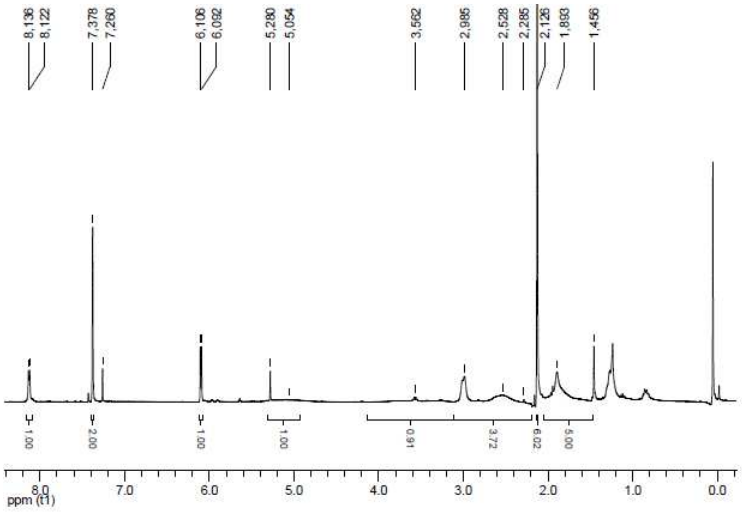

Figure S4: ^{1}H NMR spectrum of compound **10**

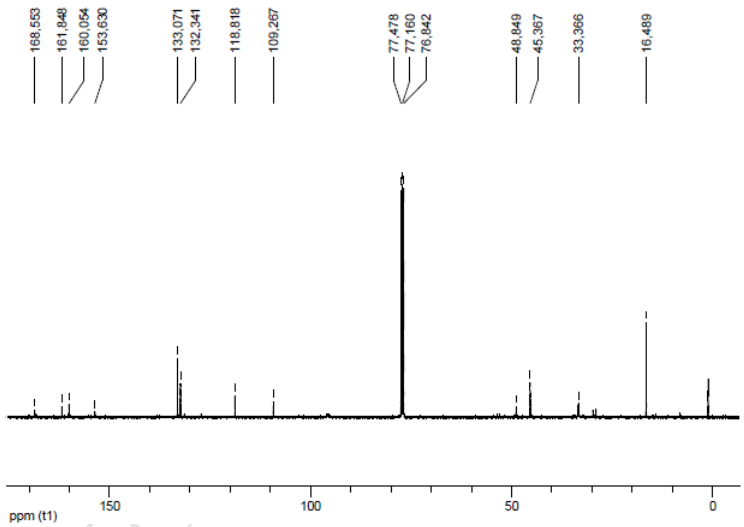

Figure S5: ^{13}C NMR spectrum of compound **10**

Figure S6: MS spectrum of compound **10**

2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(*o*-tolyl)acetamide (7b)

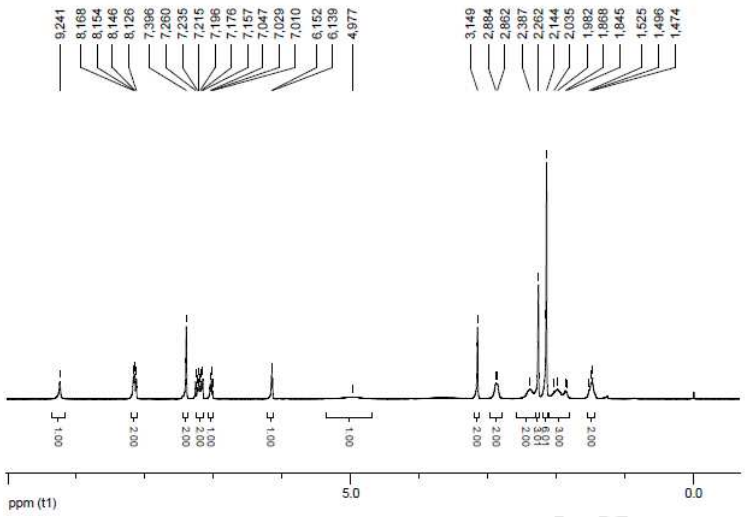

Figure S7: ^{1}H NMR spectrum of compound **7b**

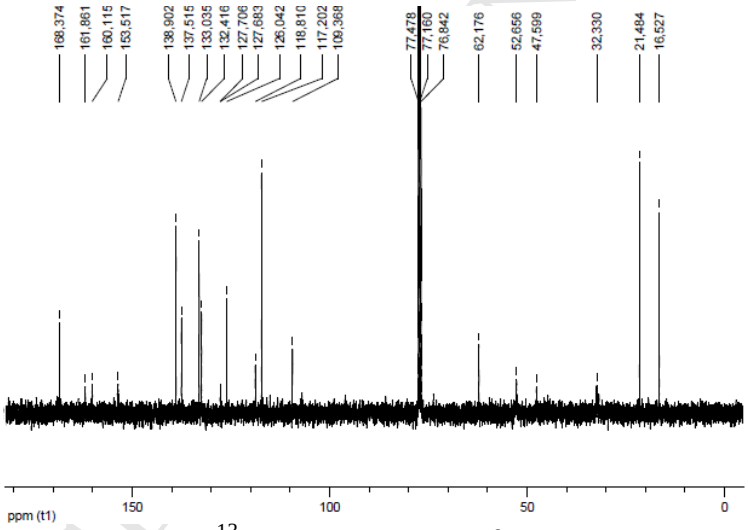

Figure S8: ^{13}C NMR spectrum of compound **7b**

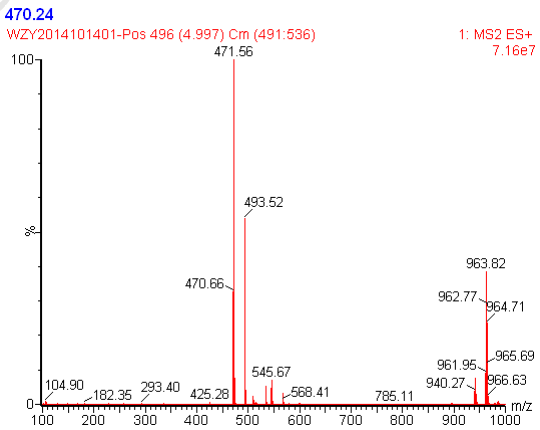

Figure S9: MS spectrum of compound **7b**

2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(3,4-dimethylphenyl)acetamide (7f)

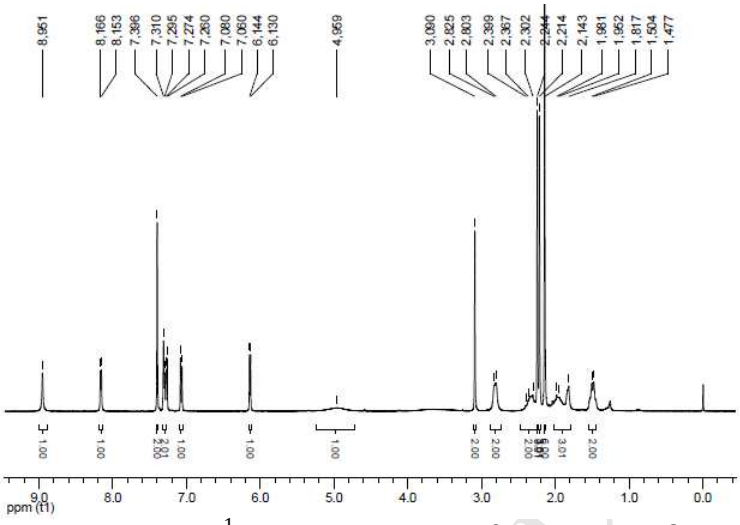

Figure S10: ^{1}H NMR spectrum of compound **7f**

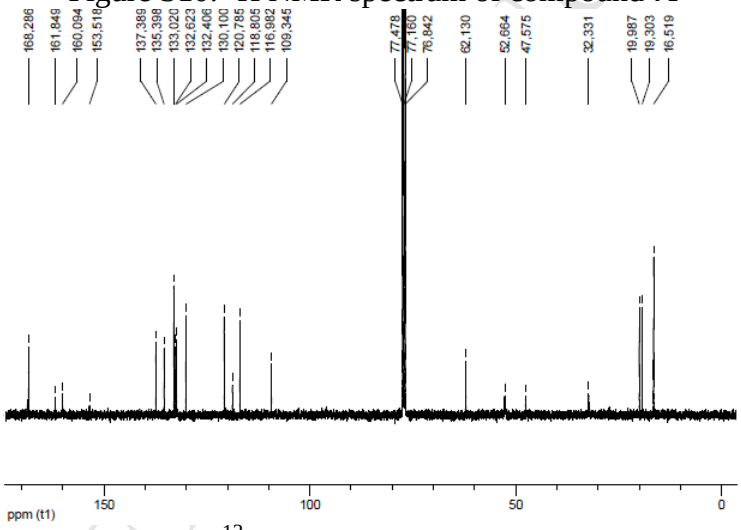

Figure S11: ^{13}C NMR spectrum of compound **7f**

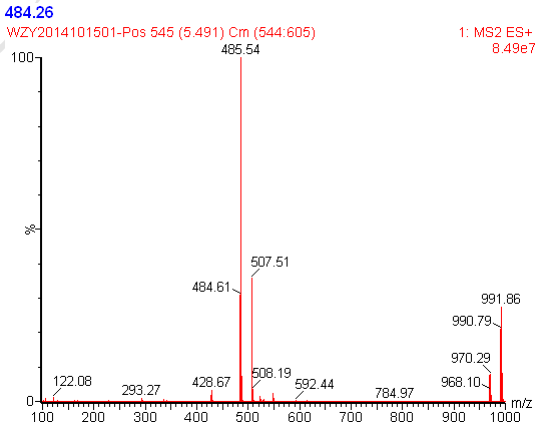

Figure S12: MS spectrum of compound **7f**

2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(4-methoxyphenyl)acetamide (7g)

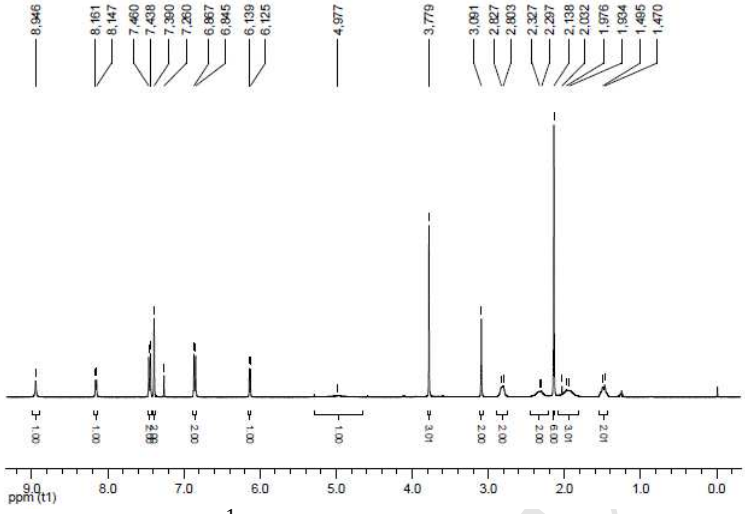

Figure S13: ^{1}H NMR spectrum of compound **7g**

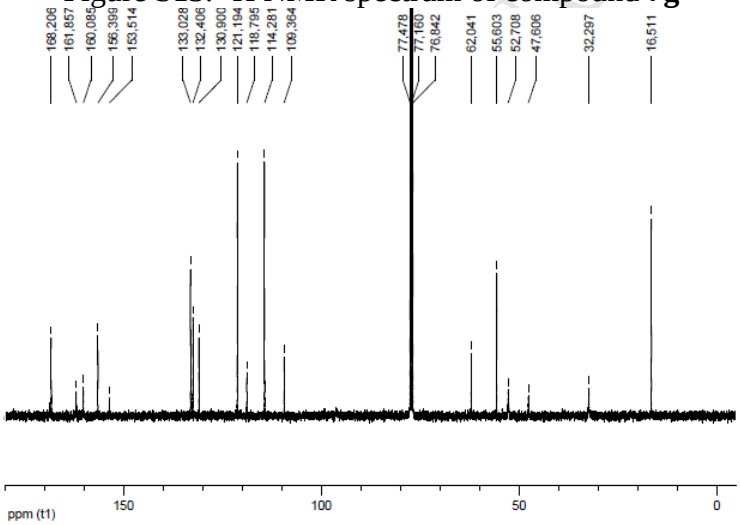

Figure S14: ^{13}C NMR spectrum of compound **7g**

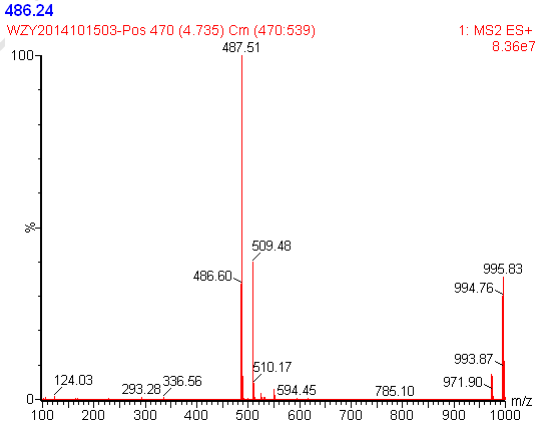

Figure S15: MS spectrum of compound **7g**

2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(2-fluorophenyl)acetamide (7h)

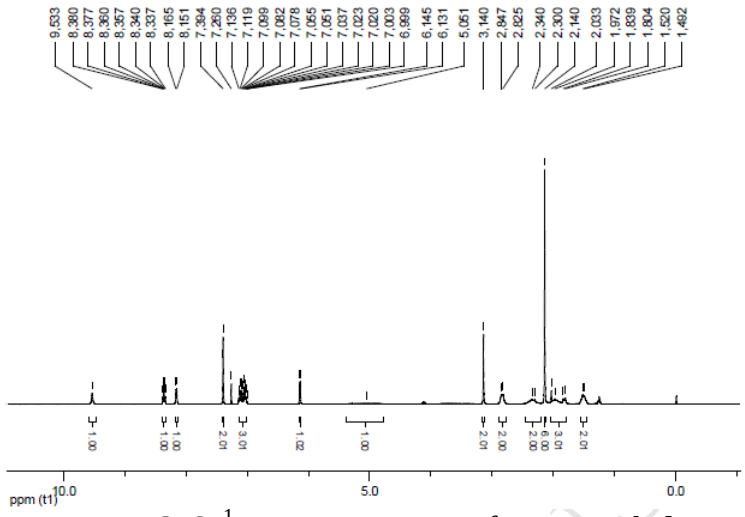

Figure S16: ^{1}H NMR spectrum of compound **7h**

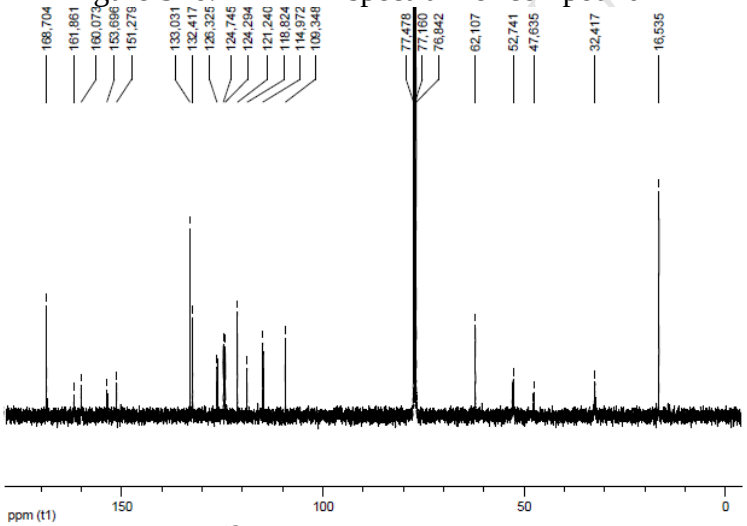

Figure S17: ^{13}C NMR spectrum of compound **7h**

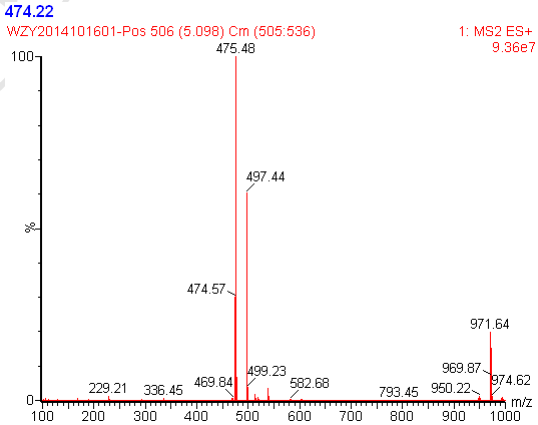

Figure S18: MS spectrum of compound **7h**

2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(3-fluorophenyl)acetamide (7i)

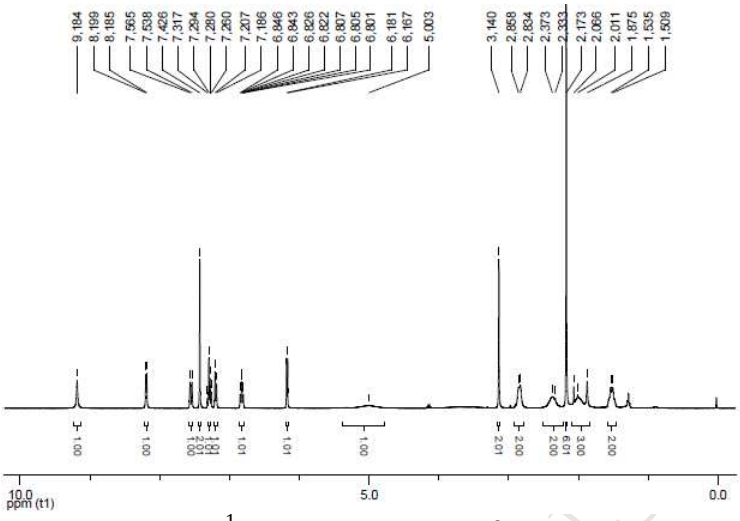

Figure S19: ^{1}H NMR spectrum of compound **7i**

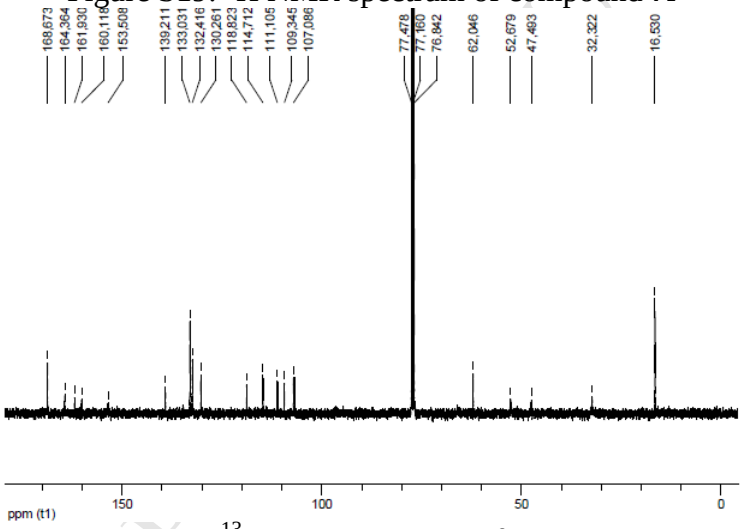

Figure S20: ^{13}C NMR spectrum of compound **7i**

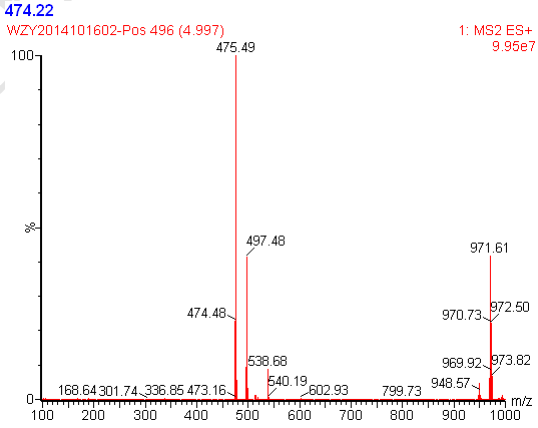

Figure S21: MS spectrum of compound **7i**

_N_-(2-Chlorophenyl)-2-(4-((4-(4-cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)acetamide (7k)

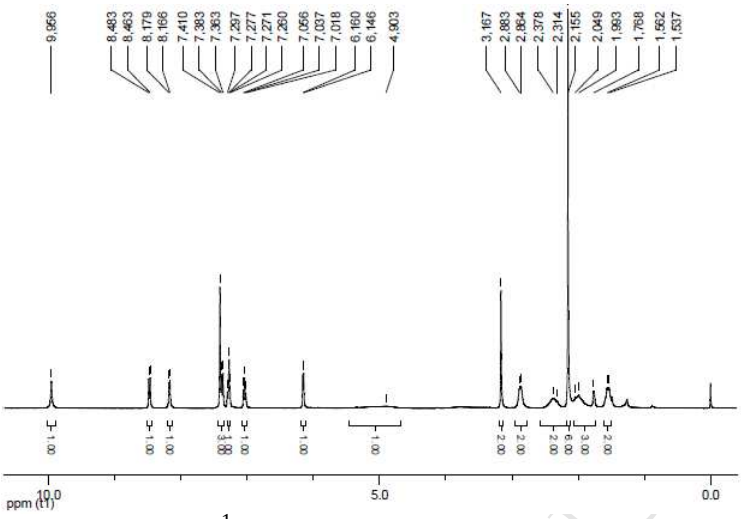

Figure S22: ^{1}H NMR spectrum of compound **7k**

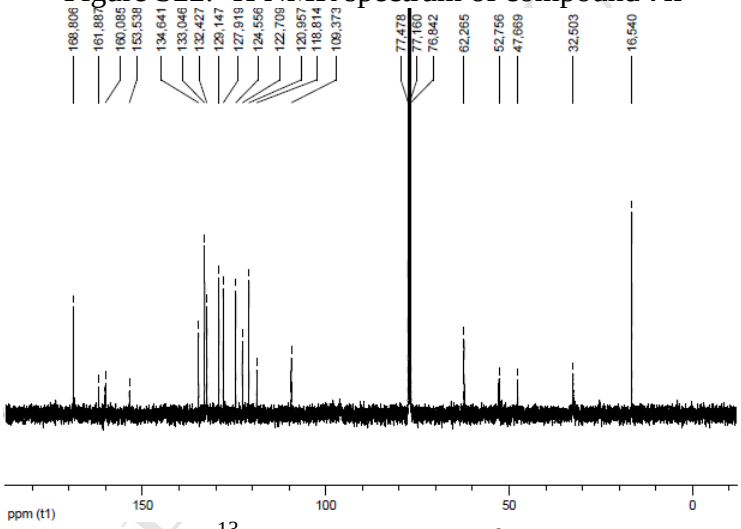

Figure S23: ^{13}C NMR spectrum of compound **7k**

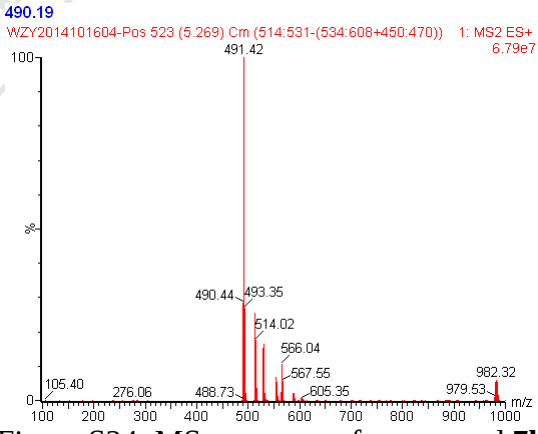

Figure S24: MS spectrum of compound **7k**

2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(3,4-dichlorophenyl)acetamide (7o)

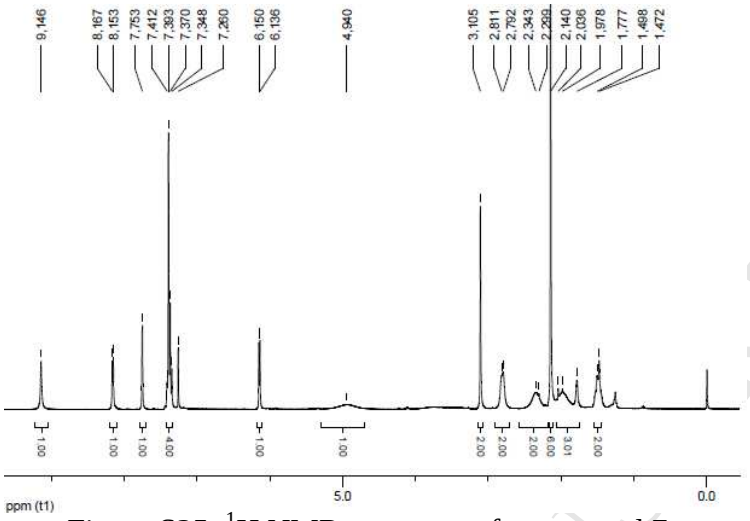

Figure S25: ^{1}H NMR spectrum of compound **7o**

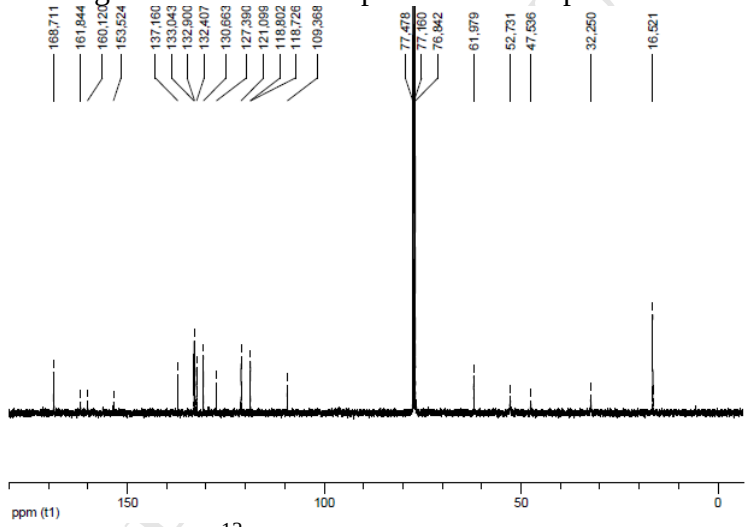

Figure S26: ^{13}C NMR spectrum of compound **7o**

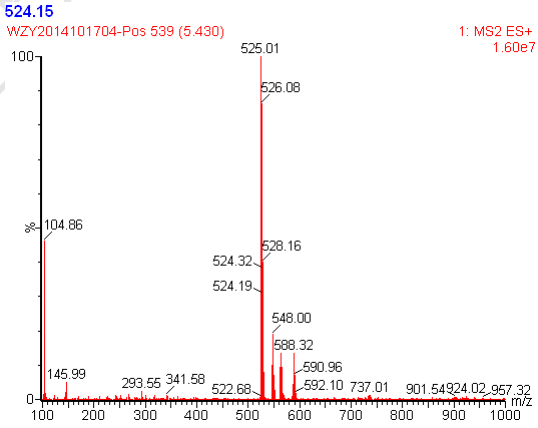

Figure S27: MS spectrum of compound **7o**

2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(2,4,6-trichlorophenyl)acetamide (7q)

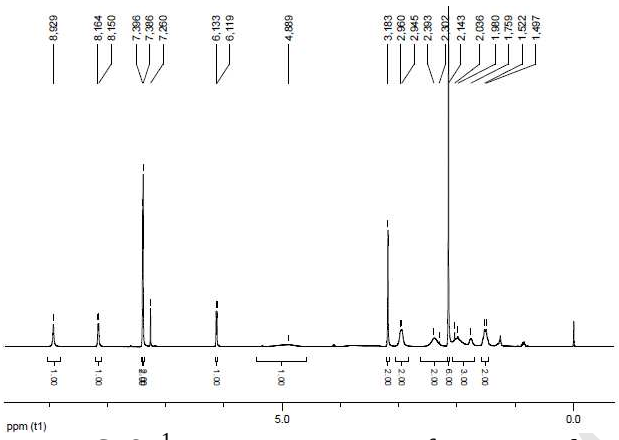

Figure S28: ^{1}H NMR spectrum of compound **7q**

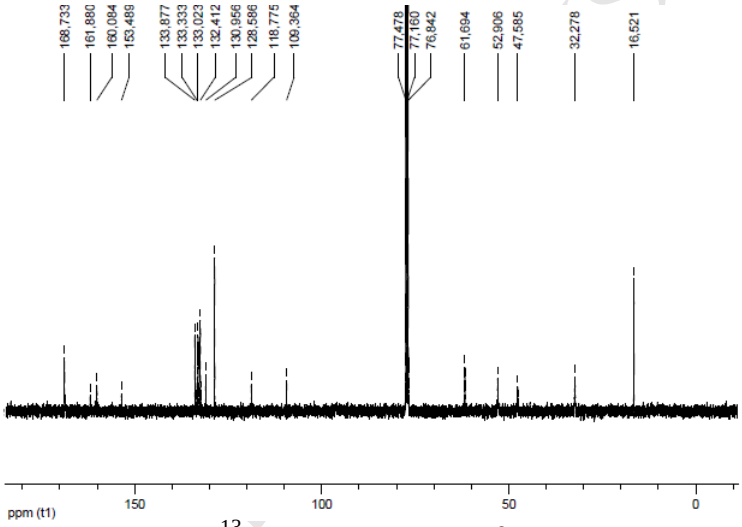

Figure S29: ^{13}C NMR spectrum of compound **7q**

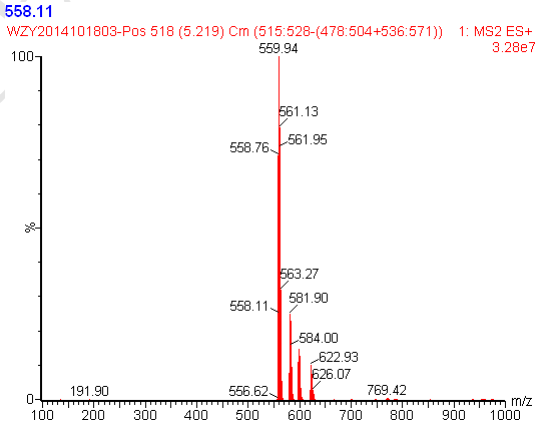

Figure S30: MS spectrum of compound **7q**

2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(4-cyano-2-fluorophenyl)acetamide (7t)

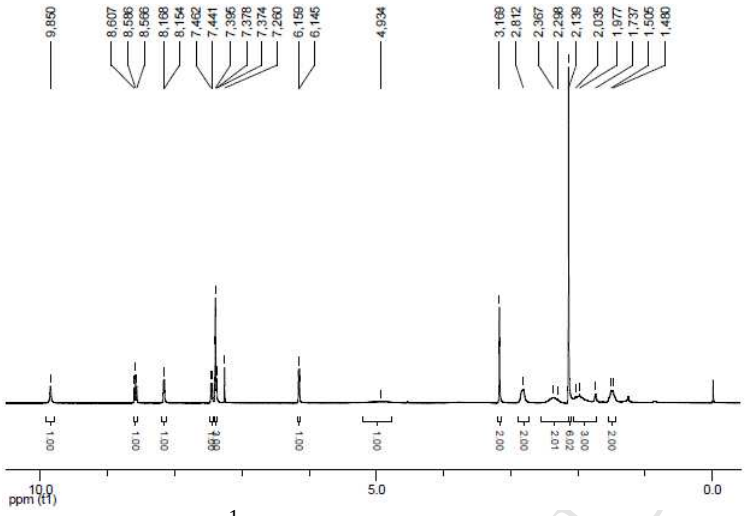

Figure S31: ^{1}H NMR spectrum of compound **7t**

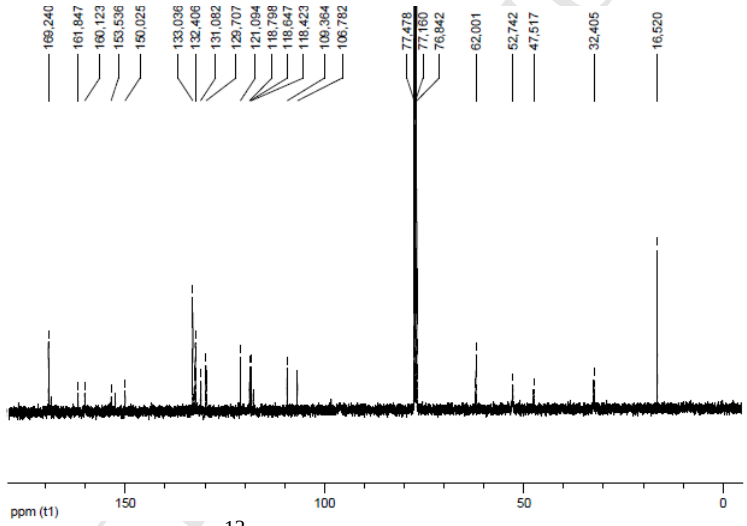

Figure S32: ^{13}C NMR spectrum of compound **7t**

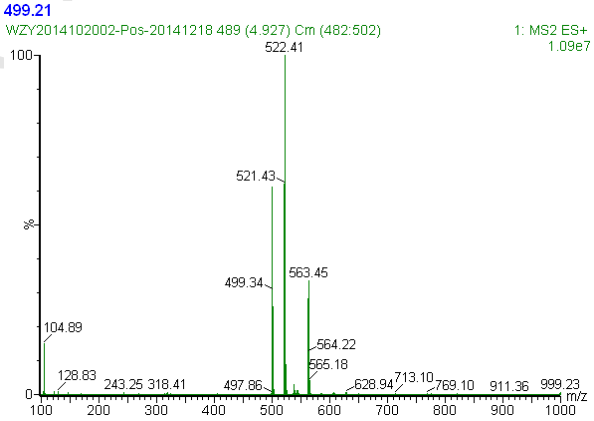

Figure S33: MS spectrum of compound **7t**

2-(4-((4-(4-Cyano-2,6-dimethylphenoxy)pyrimidin-2-yl)amino)piperidin-1-yl)-*N*-(4-nitrophenyl)acetamide (7w)

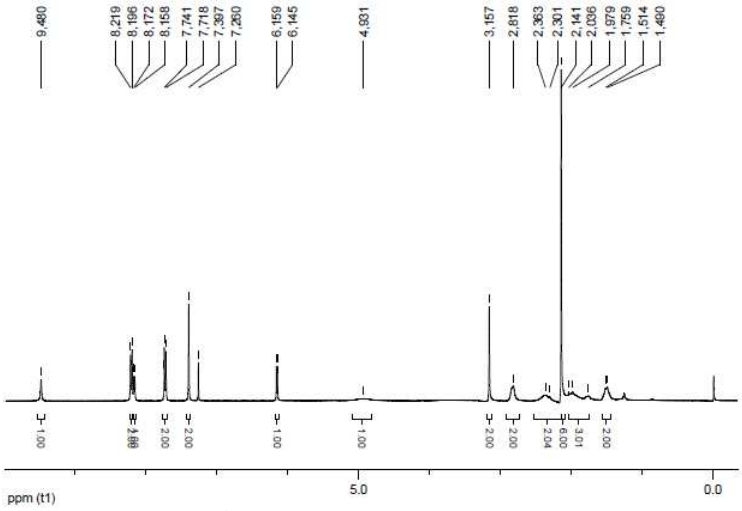

Figure S34: ^{1}H NMR spectrum of compound **7w**

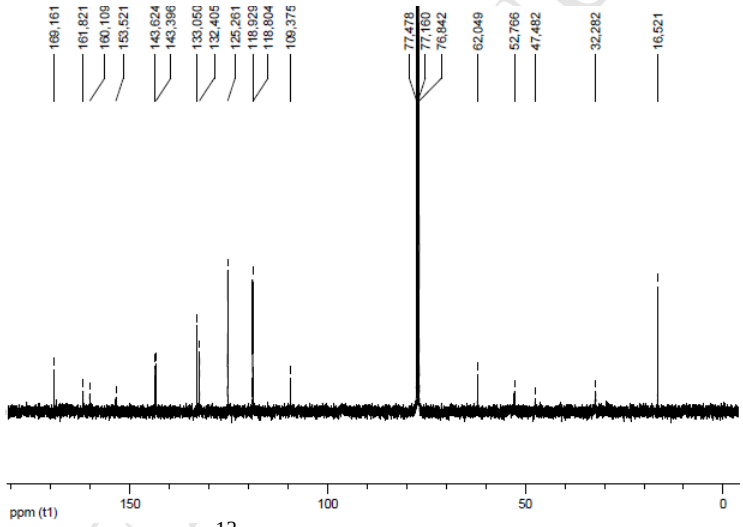

Figure S35: ^{13}C NMR spectrum of compound **7w**

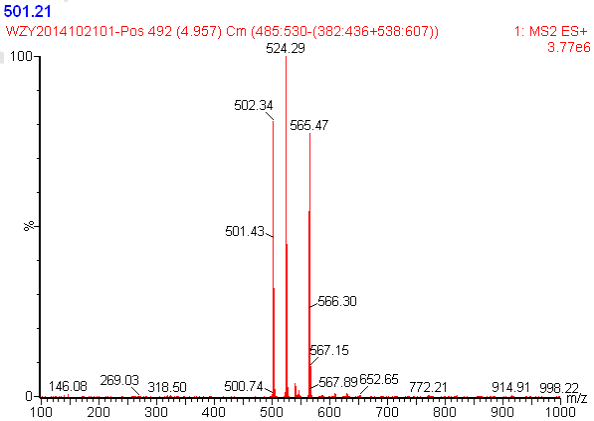

Figure S36: MS spectrum of compound **7w**