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Graphical Abstract

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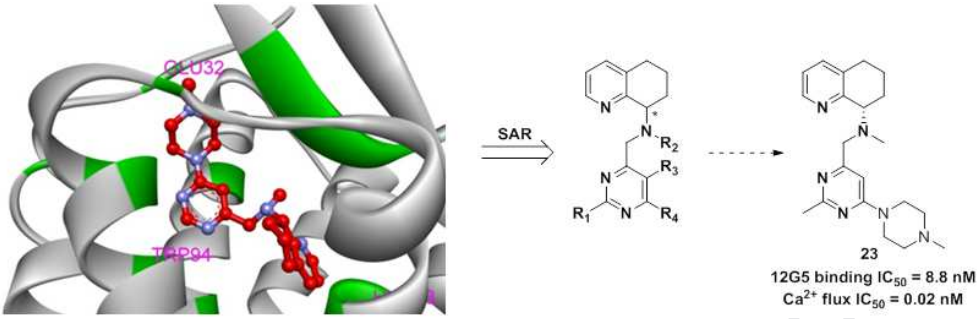

Guided by molecular docking, the reported optimization identifies a number of compounds with improved receptor binding affinity and functional activity exemplified by compound **23** (inhibition of APC-conjugate clone 12G5 for CXCR4 binding in a cell based assay: IC_{50} = 8.8 nM; inhibition of CXCL12 induced cytosolic calcium increase: IC_{50} = 0.02 nM). In addition, **23** potently inhibits CXCR4/CXLC12 mediated chemotaxis in a matrigel invasion assay, exhibits good physicochemical properties and *in vitro* safety profiles.

Structural Optimization of Aminopyrimidine-Based CXCR4 Antagonists

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Abstract: Structural optimization of aminopyrimidine-based CXCR4 antagonists is reported. The optimization is guided by molecular docking studies based on available CXCR4-small molecule crystal complex. The optimization identifies a number of compounds with improved receptor binding affinity and functional activity exemplified by compound **23** (inhibition of APC-conjugate clone 12G5 for CXCR4 binding in a cell based assay: IC_{50} = 8.8 nM; inhibition of CXCL12 induced cytosolic calcium increase: IC_{50} = 0.02 nM). In addition, compound **23** potently inhibits CXCR4/CXLC12 mediated chemotaxis in a matrigel invasion assay. Furthermore, compound **23** exhibits good physicochemical properties (MW 367, clogP 2.1, PSA 48, pKa 7.2) and *in vitro* safety profiles (marginal/moderate inhibition of CYP isozymes and hERG). These results represent significant improvement over the initial hit from scaffold hybridization and suggest that compound **23** can be used as a starting point to support lead optimization.

Keywords: Chemokine, CXCR4, antagonist, GPCR, structural optimization

Abbreviations: APC, allophycocyanin; CCD, charge coupled device; CuTC, copper(I) thiophene-2-carboxylate; CYP, cytochrome P450; DCE, 1,2-dichloroethane; DIPEA, *N*,*N*-diisopropylethylamine; DMEM, Dulbecco's modified eagle medium; DMPK, drug metabolism and pharmacokinetics; DMSO, dimethyl sulfoxide; DPBS, Dulbecco's phosphate buffered saline; EMCCD, electron-multiplying CCD; FACS, fluorescence activated cell sorter; FBS, fetal bovine serum; FLIPR, fluorescent imaging plate reader; GPCR, G protein-coupled receptor; HBSS, Hank's balanced salt solution; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; hERG, human ether-a-go-go-related gene; HI-FBS, heat inactivated FBS; HIV, human immunodeficiency virus; HLM, human liver microsomes; ICCD, intensified CCD; LDV, low dead volume microplate; LE, ligand efficiency; LLE, ligand lipophilic efficiency; MEF, mouse embryonic fibroblasts; PFA, paraformaldehyde; PK, pharmacokinetics; RLM, rat liver microsomes; rt, room temperature; SAR, structure-activity-relationship; SDF-1, stromal cell-derived factor-1; SEM, standard error measurement; SP, standard precision; THQ, tetrahydroquinoline; TIQ, tetrahydroisoquinoline; XP, extra precision.

1. Introduction

Chemokine CXCL12 (also known as stromal cell-derived factor-1, or SDF-1) and its receptor CXCR4 regulate various cellular processes including chemotaxis, survival, and proliferation [1]. CXCR4 was first identified as a coreceptor for cellular entry by T cell-tropic (X4) HIV-1 virus [2]. As a result, the majority of early research on CXCR4 antagonists focused on anti-HIV application. Small molecule CXCR4 antagonists, exemplified by AMD3100 [3], AMD11070 [4, 5], IT1t [6], MSX-122 [7], KRH3955 [8], and TIQ15 [9] (Figure 1), have been disclosed in the literature. AMD3100 (plerixafor, Figure 1) was originally developed as an anti-HIV treatment, but its poor oral bioavailability and dose-limiting toxicity prevented its further development for this chronic disease. Surprisingly, AMD3100 was found to be a potent agent in mobilizing stem cells in human and was subsequently approved by the FDA for the treatment of non-Hodgkin's lymphoma and multiple myeloma patients with hematopoietic stem cell transplantation [3]. More recently, CXCR4 was reported to play important roles in modulating cancer microenvironment by trafficking of key immune cells including T-cells, dendritic cells (DC), and myeloid derived suppressor cells (MDSC) into the tumor tissue [10]. Since majority of the immune cells were immunosuppressive (e.g., Treg, MDSC), CXCR4 was suggested to be an important culprit in evasion of immune surveillance. The clinical application of CXCR4 antagonist AMD3100 in oncology, combined with the emerging evidence of CXCR4 in modulating immune cells at the tumor microenvironment [11, 12], prompted a significant resurgent effort to discover the next generation CXCR4 antagonists.

As the first and only small molecule CXCR4 antagonist in the market place, AMD3100 is an injectable agent for short-term usage. Since autoimmune/inflammatory diseases, cancer, and HIV infection are chronic diseases, CXCR4 antagonists which are intended to be used for the aforementioned indications are expected to be administrated orally and safe for long-term use. Following this theme, AMD11070 was developed with improved oral bioavailability and safety [4]. AMD11070 progressed to clinical testing despite showing moderate CYP450 (2D6, 3A4) inhibition [13]. Other small molecule CXCR4 antagonists are in preclinical or early clinical development [14-17]. Here we wish to report our efforts toward to the development of CXCR4 antagonists which are novel, highly potent with improved *in vitro* safety profiles. Our ultimate goal is to obtain CXCR4 antagonists with good pharmacokinetic properties to support oral dosing regimen for the treatment of chronic diseases.

2. Design

Most CXCR4 antagonists contain highly basic centers. Since highly basic functional groups often lead to poor permeability, hERG/CYP inhibition and phospholipidosis [18], we set our goal to reduce basicity in our designed compounds. We previously utilized a scaffold hybridization strategy to obtain a series of CXCR4 antagonists, exemplified by compound **A** (Figure 2c) [13]. Compound **A** possesses good physicochemical properties (MW 353, clogP 2.0, pKa 6.7) and exhibits weak inhibition of hERG/CYP (racemic **A**, hERG > 30 μM, CYP inhibition at 10 μM: 3A4, 9%; 2D6 3%). In addition, compound **A** inhibits binding of APC-conjugate clone 12G5 to CXCR4 in a cell based assay (IC_{50} = 88 nM) and inhibits CXCL12 induced cytosolic calcium increase (IC_{50} = 0.73 nM) [13]. Here we report the structural optimization based on the prototypical **A**.

Molecular docking study was performed to guide our optimization efforts. Specifically, **A** was

docked into the binding pocket of CXCR4 (PDB ID: 3ODU [19] from RCSB Protein Data Bank [20]) using standard precision (SP) and extra precision (XP) modes in *Glide* docking of Schrodinger 9.0 software [21]. The detailed *Glide* docking procedures were described in the previous studies [22-24]. The computational results demonstrated that **A** had good predicted binding affinity for CXCR4 (docking score = -7.12 kcal/mol). Subsequently, the binding poses predicted by *Glide* docking and the antagonist-residues interaction spectra were depicted and analyzed. As shown in Figures 2a and 2b, **A** had tight interactions with three favorable residues including Glu32, Trp94 and His113. In addition, we found that the pyrimidine functional group of **A** was in close proximity to residues Leu41 and Tyr45. The residues within 5 Å of compound **A** in the binding pocket of CXCR4 were selected and labeled (Figure 2a). Besides residues Leu41 and Tyr45, other residues, including His281, Ser285 and Glu288, were also in close range of **A**. We hypothesized that substitutions on the pyrimidine ring can be optimized to form additional favorable interactions (hydrogen bond, electrostatic, or hydrophobic interactions) with these nearby residues (Leu41, Tyr45, His281, Ser285 and Glu288) to improve antagonistic activity of candidate compounds. Based on these observation and assessment, we formulated chemical modification/optimization plan as outlined in Figure 2c.

3. Chemistry

The general synthetic route used to prepare compounds **1-18** was outlined in Scheme 1. Ester **39** [25] was reacted with 2-methyl-2-thiopseudourea sulfate, followed by substitution with *N*-methyl piperazine to give intermediate **41**. Oxidation of **41** with Oxone and then substitution with corresponding alcohols or amines afforded intermediates **44a-k**. Direct coupling of **41** with phenylboronic acid or 1-methyl-1*H*-pyrazol-4-ylboronic acid in the presence of $Pd(PPh_3)_4$ and CuTC provided intermediates **44p** and **44q**, respectively. **44l-o** and **44r** were prepared from ester **39** in two steps as described for intermediate **41**. Compounds **1-18** were synthesized by deprotection of acetals **44a-r**, followed by reductive amination with racemic *N*-methyl-5,6,7,8-tetrahydroquinolin-8-amine [13] (*S*-isomer was used in the case of compound **18**).

The synthesis of compounds **19**, **20**, and **23-28** was performed as shown in Scheme 2. Cyclization of ester **46** with acetamidine, followed by chlorination with $POCl_3$ furnished intermediate **48**. Treatment of **49** [13] with ethylamine or cyclopropylmethylamine gave amines **50a** and **50b**, which were then alkylated with intermediate **48** and subsequently substituted by *N*-methyl piperazine to yield compounds **19** and **20**, respectively. Reaction of (*S*)-*N*-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**52**) [26] with **48** produced intermediate **53**. The synthesis of compounds **23-28** was achieved in 1-3 steps by reacting **53** with diverse amines.

Compound **21** was prepared as described in Scheme 3. Claisen condensation of ester **54** afforded ketoester **55**. Cyclization of **55** with acetamidine, followed by substitution with *N*-methyl piperazine in the presence of PyBOP gave intermediate **57**. Treatment of **57** with methylamine under thermal conditions [27] led to amine **58**, which was reacted with chloride **49** in *i*-PrOH to yield compound **21**.

The synthesis of compound **22** was performed as shown in Scheme 4. Cyclization of **59** with acetamidine, followed by substitution with *N*-Boc-piperazine in the presence of PyBOP gave intermediate **61**. Treatment of **61** with trichloroisocyanuric acid afforded chloride **62**, which was substituted with (±)-*N*-methyl-5,6,7,8-tetrahydroquinolin-8-amine to provide intermediate **63**. De-Boc protection of **63**, followed by reductive alkylation with formaldehyde led to compound **22**.

4. Results and discussion

4.1. Structure-Activity-Relationship of synthesized compounds

We used a cell based competitive binding assay to evaluate the binding affinity of compound to CXCR4 receptor. The detailed description of assay development and validation had been reported [13]. Briefly, CXCR4 expressing HPB-ALL cells were incubated with APC-conjugate clone 12G5 antibody and testing compound. The inhibition of APC-conjugate clone 12G5 antibody signal with different concentrations of compound provided inhibition curve and readout of IC_{50}. At the early stage of structural optimization, we did not synthesize the enantiomerically pure compounds associated with the chiral center on the tetrahydroquinoline (THQ) ring. We reasoned that once more potent compounds were identified, we can synthesize the chirally pure (*S*)-stereoisomer. As shown in Table 1, when the hydrogen was substituted by a methoxy group on R_1, the binding affinity was improved (**1**, 25 nM). Increasing the size of the substitution was detrimental for binding affinity (**2** and **3**, 78 and 151 nM, respectively). Substitution of methoxy to amino maintained binding affinity (**4**, 23 nM). Consistently, larger substitutions were detrimental for binding, especially for the secondary amines (compounds **5**-**11**). When methyl group was installed on the R_1 position, more than four folds of binding improvement was achieved compared to compound **A** (**12** and **A**, 12 and 54 nM, respectively). Again, decrease of binding affinity was observed when the size of the substitution increased (compounds **13**-**17**). Lastly, a strong electronwithdrawing trifluoromethyl led to decrease of binding affinity.

In order to examine the observed differences in binding activity, the two most potent CXCR4 antagonists, compounds **4** and **12** were docked into the binding site of CXCR4 (PDB ID: 3ODU) using the XP mode in *Glide* docking. The computational results demonstrated that compounds **4** (docking score = -8.26 kcal/mol) and **12** (docking score = -8.91 kcal/mol) can form additional interactions with the five nearby residues in the binding site of CXCR4 as expected (Figure 3) and reserved the tight interactions with the Glu32, Trp94 and His113 residues as identified in compound **A.** The amine group in the R_1 position of compound **4** can form hydrogen bonds with residues Ser285 and Glu288 (Figure 3b) and the methyl group in the R_1 position of compound **12** can interact with residue Leu41 (alkyl hydrophobic interaction, Figure 3c). Based on these findings, we found that chemical modifications after rational drug design using molecular docking in the R_1 position of the pyrimidine on compound **A** are reasonably reliable.

Having established methyl as the best tentative substitution on R_1, we decided to explore modifications on R_2 and R_3 positions. Moderate increase of size on R_2 was well tolerated, but provided no significant improvement on binding affinity (**19** and **20**, 17 and 51 nM, respectively). Similarly, small substitutions on R_3 led to slight decrease in binding affinity (**21** and **22**, 12 and 33 nM, respectively). Overall, the structure-activity data was consistent with the molecular docking study, as all positions modified thus far were in the helical cavity which may not tolerate big structural change.

We next focused on exploration of R_4. Since R_4 is solvent-exposed according to our docking study, we expect more tolerance to structure diversity. At this stage, we had identified the preferred substitutions on R_1, R_2, and R_3, and the best compounds showed binding affinity at low nM range. We therefore decided to synthesize the chirally pure (*S*)-stereoisomers. In agreement with literature reports and our own data [4, 13], the (*S*)-stereoisomer **23** (8.8 nM) was more potent than the racemic **12** (12 nM). As shown in Table 2, increasing the size of capping group on the terminal nitrogen of the piperazine led to decrease of binding affinity to CXCR4 (compounds **24**-**26**). Addition of methyl group

to the piperazine was well tolerated, resulting in compounds with equal or slightly decreased affinity compared with compound **23** (compounds **27**-**31**). Ring closure to form a bicyclic system did not help to improve binding affinity (**32** and **33**). Extension of the nitrogen out of the piperazine ring and addition of an extra nitrogen both led to decrease of binding affinity (**34** and **35**). Open chain analogues with different linker length were well tolerated (**36** and **37**) but removal of the basic nitrogen completely abolished binding (**38**, > 10,000 nM). In summary, R_4 could tolerate diverse functional groups with different size, rigidity, stereoisomeric configuration. However, the most important pharmacological element remained to be the basic nitrogen.

Collectively, optimization on R_1, R_2, R_3, and R_4 led to numerous compounds with improved binding affinity (e.g., compounds **23**, **29**, **31**, and **33**, 8.8, 8.3, 8.3, and 18 nM, respectively). We carefully controlled key physicochemical properties in a favorable range (e.g., clogP 2-3; pKa 6-8) throughout the structural modification. Compounds with moderate clogP and pKa are less likely to encounter safety issues (CYP/hERG inhibition) and may have better chance to be progressed further [18, 28]. Since compounds **23**, **29**, **31**, and **33** were similar in structure and showed comparable binding affinity, we decided to use LE (ligand efficiency) and LLE (ligand lipophilic efficiency) as stratification criterion to choose a compound for further proof of concept evaluation. Compound **23** had the lowest molecular weight/lipophilicity and was therefore selected as the prototypical compound for functional testing.

4.2. Functional evaluation of lead compound 23

One of the hallmarks of CXCR4 activation by CXCL12 is the transient increase of cytosolic calcium concentration. Inhibition of the CXCL12-induced calcium signal can be used to evaluate functional activity of CXCR4 antagonists. The calcium mobilization assay can be efficiently performed by FLIPR Tetra system [29]. Since compound **23** demonstrated high binding affinity to CXCR4 in the 12G5 competitive binding assay, we tested compound **23** for its functional activity in the FLIPR Tetra based calcium mobilization assay. The detailed experimental procedure was reported previously [13].

Compound **23** proved to be a potent functional CXCR4 antagonist (IC_{50} = 0.02 nM) while the positive control AMD3100 was much less potent (IC_{50} = 6 nM) under the same assay conditions (Figure 4a). We further assessed the preliminary functional activities/specificities of compound **23** by testing it in CXCR1 (within the CXCR sub-family) and CCR6 (outside of the CXCR sub-family) functional assays. Compound **23** proved to be inactive up to 10,000 nM (Figure 4b, c). Compound **23** binds with CXCR4 with high affinity (IC_{50} = 8.8 nM in the 12G5 competitive binding assay) and inhibits CXCR4 function with excellent potency (IC_{50} = 0.02 nM in the FLIPR Tetra based calcium mobilization assay). The divergence of the binding and functional data might reflect the interaction mechanism of compound **23** with CXCR4 receptor. It is possible that compound **23** abolishes CXCL12 induced calcium flux (D97, E288 mediated) more efficiently than it blocks engagement of 12G5 with CXCR4 (ECL-2 mediated) [30]. However, crystal/mutational data are needed to draw conclusion.

We next investigated compound **23** in the matrigel invasion assay to evaluate its inhibition of CXCL12/CXCR4 mediated chemotaxis. MDA-MB-231 cells which naturally express CXCR4 and compound **23** (100 nM) were added to the upper chamber. Human CXCL12 (200 ng/mL) was added to the lower chamber. The inhibition of cell invasion with compound **23** and AMD3100 was calculated by comparing to the cell invasion without treatment (Figure 5a, b). In agreement with the 12G5 binding and the calcium mobilization data, compound **23** exhibited superior potency in the matrigel invasion

assay than AMD3100. Since compounds with general cellular toxicity can impede cell mobility, we further investigated the potential cytotoxicity effect of compound **23** using the cell viability assay (ATP-based Cell Titer-Glo Luminescent Cell Viability Assay). As shown in Figure 5c, compound **23** did not inhibit the proliferation of multiple human and murine cells, including MDA-MB-231 and mouse embryonic fibroblasts (MEF), at concentrations up to 1000 nM.

4.3. Preliminary in vitro safety evaluation of lead compound

Based on its excellent *in vitro* pharmacological properties, we decided to evaluate compound **23** for its in vitro safety profiles. As shown in Table 3, compound **23** displayed moderate inhibition of CYP3A4 and 2D6 at 10 μM concentration (48% and 57%, respectively), similar to these of AMD11070 (60% and 64%, respectively) [13]. In order to assess its potential cardiotoxicity, compound **23** was tested in a standard patch clamp (express) experiment for its inhibition of the human ether-a-go-go related gene (hERG) potassium channel. Again, compound **23** displayed moderate inhibition (IC_{50} = 8 μM) of hERG (positive control: cisapride, 0.03 μM). Although the *in vitro* safety window seemed to be reasonable for hERG (1000 folds based on binding affinity, over 100,000 folds based on calcium mobilization), the inhibition potency of 8 μM remained a safety concern. On the other hand, most of the published CXCR4 antagonists displayed poor to moderate pharmacokinetic profiles [6, 8, 9, 31], including low C_{max}, which suggested better tolerance for hERG inhibition. Nevertheless, future optimization must consider a balanced CXCR4 potency and safety/PK profiles. Compound **23** was found to be highly permeable (27×10^{-6} cm/s) with low efflux ratio when measured in a Caco-2 permeability assay (Table 3). In addition, compound **23** displayed moderate plasma protein binding in mouse and rat (89% and 82%, respectively) but high plasma protein binding in human (99%). Lastly, compound **23** was quickly metabolized in rat liver microsomes (Table 3).

5. Conclusion

Guided by molecular docking studies based on available CXCR4-small molecule crystal complex, the current optimization identifies a number of compounds with improved receptor binding affinity and functional activity exemplified by compound **23** (inhibition of APC-conjugate clone 12G5 for CXCR4 binding in a cell based assay: IC_{50} = 8.8 nM; inhibition of CXCL12 induced cytosolic calcium increase: IC_{50} = 0.02 nM). In addition, compound **23** potently inhibits CXCR4/CXLC12 mediated chemotaxis in a matrigel invasion assay. Furthermore, compound **23** exhibits good physicochemical properties (MW 367, clogP 2.1, PSA 48, pKa 7.2) and *in vitro* safety profiles (marginal/moderate inhibition of CYP isozymes and hERG). However, the metabolic stability in liver microsomes is suboptimal and needs to be optimized. While this work was in progress, we encountered a report from Emory University in which they presented metabolic data on their tetrahydroisoquinoline (TIQ) series of compounds [32]. They showed hydroxylation on the THQ moiety in mouse liver microsomes was the culprit for high clearance. Blockade of the metabolic soft spot on the THQ moiety shifted the metabolic liability to the TIQ moiety. Encouraged by their results, we are currently working on structural modification on the THQ moiety on our scaffold and the results will be reported in due course.

6. Experimental protocols

6.1. Chemistry

All reagents and solvents were used without any further purification. Reactions were monitored by thin-layer chromatography or by Agilent 1100 LC/MSD Trap SL version Mass Spectrometer. ^{1}H and ^{13}C NMR spectra were performed on Varian 400 MHz or 600 MHz spectrometers with tetramethylsilane as an internal reference. HRMS analysis was recorded on an Agilent 6540 UHD Accurate-Mass Q-TOF LC/MS.

*6.1.1. 6-(Dimethoxymethyl)-2-(methylthio)pyrimidin-4-ol (**40**)*

A mixture of **39** (3.0 g, 17 mmol), 2-methyl-2-thiopseudourea sulfate (9.5 g, 34 mmol) in100 mL of H_2O was added K_2CO_3 (10 g, 76 mmol). The mixture was stirred at room temperature overnight and acidified pH to 5 by AcOH. The solution was extracted with dichloromethane (30 mL x 3). The combined organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 100/1) to give the desired product (3.4 g, 92%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.79 (s, 1H), 6.46 (s, 1H), 5.06 (s, 1H), 3.39 (s, 6H), 2.60 (s, 3H). MS (ESI/APCI) m/z 216.9 $[M+H]^+$.

*6.1.2. 4-(Dimethoxymethyl)-6-(4-methylpiperazin-1-yl)-2-(methylthio)pyrimidine (**41**)*

To a solution of **40** (3.4 g, 16 mmol), Et_3N (16 g, 160 mmol) and *N*-methyl piperazine (4.0 g, 40 mmol) in 100 mL of MeCN was added PyBOP (9.0 g, 17 mmol). The mixture was stirred at reflux overnight. The reaction mixture was quenched with saturated $NaHCO_3$ aqueous solution (200 mL) and extracted with dichloromethane (200 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 100/3) to give the product (2.8 g, 59%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.44 (s, 1H), 5.06 (s, 1H), 3.69 (s, 4H), 3.40 (s, 6H), 2.50 (s, 3H), 2.46 (t, *J* = 5.0 Hz, 4H), 2.34 (s, 3H). MS (ESI/APCI) m/z 298.8 $[M+H]^+$.

*6.1.3. 4-(Dimethoxymethyl)-6-(4-methylpiperazin-1-yl)-2-(methylsulfonyl)pyrimidine (**42**)*

To a solution of **41** (2.8 g, 9.4 mmol) in THF (90 mL) and H_2O (4.5 mL) was added Oxone (6.7 g, 11 mmol) at room temperature. The mixture was stirred at room temperature for 4 h. The reaction mixture was quenched with saturated $NaHCO_3$ aqueous solution (200 mL) and extracted with ethyl acetate (200 mL). The organic layer was dried over Na_2SO_4 and concentrated to give the desired product (2.4 g, crude) as a yellow oil, which was used directly in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.85 (s, 1H), 5.17 (s, 1H), 4.00-3.60 (m, 4H), 3.42 (s, 6H), 3.29 (s, 3H), 2.53-2.47 (m, 4H), 2.35 (s, 3H). MS (ESI/APCI) m/z 330.8 $[M+H]^+$.

*6.1.4. General procedure for the synthesis of **43a-e***

To a mixture of **39** (1.7 mmol, 1.0 eq.), corresponding amidine (2.0 eq.) in 10 mL of H_2O was added K_2CO_3 (4.5 eq.). The mixture was stirred at room temperature overnight and acidified pH to 5 by AcOH. The solution was extracted with dichloromethane (30 mL * 3). The combined organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 50/1) to give the desired product.

*6-(Dimethoxymethyl)-2-methylpyrimidin-4-ol (**43a**)*

Compound **43a** was obtained as a white solid (yield 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.12 (s, 1H), 6.56 (s, 1H), 5.09 (s, 1H), 3.38 (s, 6H), 2.51 (s, 3H). MS (ESI/APCI) m/z 185.0 $[M+H]^+$.

*6-(Dimethoxymethyl)-2-ethylpyrimidin-4-ol (**43b**)*

Compound **43b** was obtained as a colorless oil (yield 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.57 (s, 1H), 5.10 (s, 1H), 3.39 (s, 6H), 2.75 (q, *J* = 7.6 Hz, 2H), 1.35 (t, *J* = 7.6 Hz, 3H). MS (ESI/APCI) m/z 198.9 $[M+H]^+$.

*6-(Dimethoxymethyl)-2-isopropylpyrimidin-4-ol (**43c**)*

Compound **43c** was obtained as a white solid (yield 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.28 (s, 1H), 6.57 (s, 1H), 5.10 (s, 1H), 3.39 (s, 6H), 3.03-2.93 (m, 1H), 1.34 (d, *J* = 7.2 Hz, 6H).

*2-Cyclopropyl-6-(dimethoxymethyl)pyrimidin-4-ol (**43d**)*

Compound **43d** was obtained as a white solid (yield 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.00 (s, 1H), 6.49 (s, 1H), 5.00 (s, 1H), 3.37 (s, 6H), 1.98-1.87 (m, 1H), 1.28-1.22 (m, 2H), 1.17-1.03 (m, 2H). MS (ESI/APCI) m/z 210.9 $[M+H]^+$.

*6-(Dimethoxymethyl)-2-(trifluoromethyl)pyrimidin-4-ol (**43e**)*

Compound **43e** was obtained as a yellow solid (yield 36%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (s, 1H), 5.22 (s, 1H), 3.42 (s, 6H). MS (ESI/APCI) m/z 238.8 $[M+H]^+$.

*6.1.5. General procedure for the synthesis of **44a** and **44b***

To a solution of **42** (0.61 mmol, 1.0 eq.) in 5 mL of MeOH or EtOH was added MeONa or EtONa (5.0 eq.). The mixture was stirred at reflux overnight. The reaction mixture was quenched with saturated $NaHCO_3$ aqueous solution and extracted with dichloromethane. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 100/1 to 100/3) to give the desired product.

*4-(Dimethoxymethyl)-2-methoxy-6-(4-methylpiperazin-1-yl)pyrimidine (**44a**)*

Compound **44a** was obtained as a yellow oil (yield 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.71 (s, 1H), 6.38 (s, 1H), 5.02 (s, 1H), 4.02 (s, 3H), 3.40 (s, 6H).

*4-(Dimethoxymethyl)-2-ethoxy-6-(4-methylpiperazin-1-yl)pyrimidine **(44b)***

Compound **44b** was obtained as a yellow oil (yield 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.42 (s, 1H), 5.04 (s, 1H), 4.35 (q, *J* = 7.2 Hz, 2H), 3.68 (s, 4H), 3.40 (s, 6H), 2.45 (t, *J* = 5.0 Hz, 4H), 2.32 (s, 3H), 1.38 (t, *J* = 7.0 Hz, 3H). MS (ESI/APCI) m/z 296.9 $[M+H]^+$.

*6.1.6. 4-(Dimethoxymethyl)-2-isopropoxy-6-(4-methylpiperazin-1-yl)pyrimidine (**44c**)*

To a solution of **42** (240 mg, 0.73 mmol) in THF (5 mL) and *i*-PrOH (2 mL) was added NaH (60% dispersion in mineral oil, 35 mg, 0.87 mmol). The mixture was stirred at 80°C for 2 h. The reaction mixture was quenched with saturated $NaHCO_3$ aqueous solution and extracted with dichloromethane. The organic layer was dried over Na_2SO_4 and concentrated to give the product (220 mg, 97%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.39 (s, 1H), 5.28-5.18 (m, 1H), 5.03 (s, 1H), 3.68 (s, 4H), 3.41 (s, 6H), 2.45 (t, *J* = 5.0 Hz, 4H), 2.33 (s, 3H), 1.35 (d, *J* = 6.2 Hz, 6H). MS (ESI/APCI) m/z 310.9 $[M+H]^+$.

*6.1.7. General procedure for the synthesis of **44d-k***

A mixture of **42** (1.4 mmol, 1.0 eq.) in 5 mL of corresponding amine/THF (2 M) was stirred at reflux overnight in a sealed tube. The reaction mixture was quenched with saturated $NaHCO_3$ aqueous solution and extracted with dichloromethane. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 100/1 to 100/3) to give the desired product.

*4-(Dimethoxymethyl)-6-(4-methylpiperazin-1-yl)pyrimidin-2-amine (**44d**)*

Compound **44d** was obtained as a yellow oil (yield 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.20 (s, 1H), 5.02 (s, 1H), 4.77 (s, 2H), 3.63 (s, 4H), 3.38 (s, 6H), 2.43 (t, *J* = 5.0 Hz, 4H), 2.32 (s, 3H). MS (ESI/APCI) m/z 267.9 $[M+H]^+$.

*4-(Dimethoxymethyl)-N-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-2-amine (**44e**)*

Compound **44e** was obtained as a yellow oil (yield 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.12 (s, 1H), 5.01 (s, 1H), 3.67 (s, 4H), 3.39 (s, 6H), 2.94 (d, *J* = 5.2 Hz, 3H), 2.45 (t, *J* = 5.2 Hz, 4H), 2.33 (s, 4H).

*4-(Dimethoxymethyl)-N-ethyl-6-(4-methylpiperazin-1-yl)pyrimidin-2-amine (**44f**)*

Compound **44f** was obtained as a yellow oil (yield 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.11 (s, 1H), 5.00 (s, 1H), 4.87 (s, 1H), 3.65 (s, 4H), 3.43-3.35 (m, 8H), 2.44 (t, *J* = 5.0 Hz, 4H), 2.33 (s, 3H), 1.18 (t, *J* = 7.2 Hz, 3H).

*4-(Dimethoxymethyl)-N-isopropyl-6-(4-methylpiperazin-1-yl)pyrimidin-2-amine (**44g**)*

Compound **44g** was obtained as a yellow oil (340 mg, crude). This material was used directly in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.08 (s, 1H), 5.75 (br s, 1H), 5.03 (s, 1H), 4.12–3.98 (m, 1H), 3.67 (s, 4H), 3.38 (s, 6H), 2.46 (s, 4H), 2.32 (s, 3H), 1.19 (d, *J* = 6.0 Hz, 6H). MS (ESI/APCI) m/z 309.9 $[M+H]^+$.

*4-(Dimethoxymethyl)-N,N-dimethyl-6-(4-methylpiperazin-1-yl)pyrimidin-2-amine (**44h**)*

Compound **44h** was obtained as a yellow oil (yield 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.07 (s, 1H), 4.99 (s, 1H), 3.63 (s, 4H), 3.40 (s, 6H), 3.13 (s, 6H), 2.44 (s, 4H), 2.32 (s, 3H).

*2-((4-(Dimethoxymethyl)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)(methyl)amino)ethan-1-ol (**44i**)*

Compound **44i** was obtained as a yellow oil (yield 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.11 (s, 1H), 5.00 (s, 1H), 3.84 (t, *J* = 4.6 Hz, 2H), 3.71 (t, *J* = 4.6 Hz, 2H), 3.68-3.60 (m, 4H), 3.38 (s, 6H), 3.17 (s, 3H), 2.44 (t, *J* = 5.2 Hz, 4H), 2.32 (s, 3H). MS (ESI/APCI) m/z 325.9 $[M+H]^+$.

*4-(4-(Dimethoxymethyl)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)morpholine (**44j**)*

Compound **44j** was obtained as a yellow oil (yield 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.14 (s, 1H), 4.98 (s, 1H), 3.74 (s, 8H), 3.63 (s, 4H), 3.40 (s, 6H), 2.44 (t, *J* = 5.0 Hz, 4H), 2.32 (s, 3H).

*4-(Dimethoxymethyl)-6-(4-methylpiperazin-1-yl)-2-(pyrrolidin-1-yl)pyrimidine (**44k**)*

Compound **44k** was obtained as a yellow oil (yield 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.09 (s, 1H), 5.01 (s, 1H), 3.69-3.61 (m, 4H), 3.58-3.50 (m, 4H), 3.41 (s, 6H), 2.44 (t, *J* = 5.0 Hz, 4H), 2.32 (s, 3H), 1.94-1.88 (m, 4H).

*6.1.8. General procedure for the synthesis of **44p** and **44q***

A mixture of **41** (1.0 mmol, 1.0 eq.), phenylboronic acid or 1-methyl-*1H*-pyrazole-4-boronicacid (2.0 eq.), copper(I) thiophene-2-carboxylate (2.6 eq.), and $Pd(PPh_3)_4$ (0.1 eq.) in 20 mL of THF was stirred at 80°C for 12 h under N_2. The resulting solution was evaporated to remove most of THF. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 100/1.5) to give the desired product.

*4-(Dimethoxymethyl)-6-(4-methylpiperazin-1-yl)-2-phenylpyrimidine (**44p**)*

Compound **44p** was obtained as a colorless oil (yield 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49-8.28 (m, 2H), 7.44-7.43 (m, 3H), 6.70 (s, 1H), 5.24 (s, 1H), 3.81 (s, 4H), 3.46 (s, 6H), 2.59-2.42 (m, 4H), 2.36 (s, 3H). MS (ESI/APCI) m/z 328.9$[M+H]^+$.

*4-(Dimethoxymethyl)-2-(1-methyl-1H-pyrazol-4-yl)-6-(4-methylpiperazin-1-yl)pyrimidine (**44q**)*

Compound **44q** was obtained as a colorless oil (yield 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 8.04 (s, 1H), 6.57 (s, 1H), 5.15 (s, 1H), 3,93 (s, 3H), 3.67-3.77 (m, 4H), 3.42 (s, 6H), 2.53-2.43 (m, 4H), 2.34 (s, 3H). MS (ESI/APCI) m/z 332.9 $[M+H]^+$.

*6.1.9. General procedure for the synthesis of **44l-o** and **44r***

To a solution of **43a/b/c/d/e** (0.6 mmol, 1.0 eq.), Et_3N (6.0 eq.) and *N*-methyl piperazine (1.5 eq.) in 10 mL of MeCN was added PyBOP (1.1 eq.). The mixture was stirred at reflux overnight. The reaction mixture was quenched with saturated $NaHCO_3$ aqueous solution and extracted with dichloromethane. The organic layer was dried over Na_2SO_4 and concentrated to give the desired product, which was used directly in the next step without further purification.

*4-(Dimethoxymethyl)-2-methyl-6-(4-methylpiperazin-1-yl)pyrimidine (**44l**)*

Compound **44l** was obtained as a yellow oil (140 mg, crude). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.58

(s, 1H), 5.13 (s, 1H), 3.71 (s, 4H), 3.39(s, 6H), 2.52 (s, 3H), 2.49 (s, 4H), 2.35 (s, 3H). MS (ESI/APCI) m/z 266.9 $[M+H]^+$.

*4-(Dimethoxymethyl)-2-ethyl-6-(4-methylpiperazin-1-yl)pyrimidine (**44m**)*

Compound **44m** was obtained as a yellow oil (460 mg, crude). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.59 (s, 1H), 5.14 (s, 1H), 3.73 (s, 4H), 3.40 (s, 6H), 2.78 (q, *J* = 7.6 Hz, 2H), 2.58-2.44 (m, 4H), 2.36 (s, 3H), 1.29 (t, *J* = 7.6 Hz, 3H). MS (ESI/APCI) m/z 280.9 $[M+H]^+$.

*4-(Dimethoxymethyl)-2-isopropyl-6-(4-methylpiperazin-1-yl)pyrimidine (**44n**)*

Compound **44n** was obtained as a brown oil (270 mg, crude). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.57 (s, 1H), 5.13 (s, 1H), 3.71 (s, 4H), 3.40 (s, 6H), 3.08-2.97 (m, 1H), 2.54-2.42 (m, 4H), 2.34 (s, 3H), 1.26 (d, *J* = 6.8 Hz, 6H). MS (ESI/APCI) m/z 294.9 $[M+H]^+$.

*2-Cyclopropyl-4-(dimethoxymethyl)-6-(4-methylpiperazin-1-yl)pyrimidine (**44o**)*

Compound **44o** was obtained as a yellow oil (380 mg, crude). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.54 (s, 1H), 5.13 (s, 1H), 3.68 (s, 4H), 3.40 (s, 6H), 2.50 (s, 4H), 2.36 (s, 3H), 2.10 (s, 1H), 1.06 (s, 2H), 0.98-0.92 (m, 2H). MS (ESI/APCI) m/z 292.9 $[M+H]^+$.

*4-(Dimethoxymethyl)-6-(4-methylpiperazin-1-yl)-2-(trifluoromethyl)pyrimidine (**44r**)*

Compound **44r** was obtained as a yellow oil (800 mg, crude). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84 (s, 1H), 5.18 (s, 1H), 3.76 (s, 4H), 3.42 (s, 6H), 2.50 (s, 4H), 2.35 (s, 3H). MS (ESI/APCI) m/z 320.8 $[M+H]^+$.

*6.1.10. General procedure for the synthesis of **45l-o** and **45r***

A mixture of **44l/m/n/o/r** (1.0 eq.) in 5 mL of 20% H_2SO_4 was stirred at reflux overnight. The reaction mixture was alkalified pH to 9 by saturated $NaHCO_3$ aqueous solution and extracted with dichloromethane (30 mL). The organic layer was dried over Na_2SO_4 and concentrated to give the desired product.

*2-Methyl-6-(4-methylpiperazin-1-yl)pyrimidine-4-carbaldehyde (**45l**)*

Compound **45l** was obtained as a yellow oil (yield 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.88 (s, 1H), 6.88 (s, 1H), 3.74 (s, 4H), 2.59 (s, 3H), 2.48 (t, *J* = 5.2 Hz, 4H), 2.34 (s, 3H).

*2-Ethyl-6-(4-methylpiperazin-1-yl)pyrimidine-4-carbaldehyde (**45m**)*

Compound **45m** was obtained as a brown oil (yield 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (s, 1H), 6.88 (s, 1H), 3.75 (s, 4H), 2.84 (q, *J* = 7.6 Hz, 2H), 2.53-2.43 (m, 4H), 2.34 (s, 3H), 1.33 (t, *J* = 7.4 Hz, 3H).

*2-Isopropyl-6-(4-methylpiperazin-1-yl)pyrimidine-4-carbaldehyde (**45n**)*

Compound **45n** was obtained as a yellow solid (yield 37%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (s, 1H), 6.87 (s, 1H), 3.75 (m, 4H), 3.16-2.98 (m, 1H), 2.53-2.44 (m, 4H), 2.34 (s, 3H), 1.30 (d, *J* = 7.2 Hz, 6H).

*2-Cyclopropyl-6-(4-methylpiperazin-1-yl)pyrimidine-4-carbaldehyde (**45o**)*

Compound **45o** was obtained as a yellow solid (yield 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.86 (s, 1H), 6.83 (s, 1H), 3.69 (s, 4H), 2.50-2.41 (m, 4H), 2.33 (s, 3H), 2.17-2.08 (m, 1H), 1.12-1.07 (m, 2H), 1.03-0.95 (m, 2H).

*6-(4-Methylpiperazin-1-yl)-2-(trifluoromethyl)pyrimidine-4-carbaldehyde (**45r**)*

Compound **45r** was obtained as a yellow solid (yield 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.94 (s, 1H), 7.13 (s, 1H), 3.75 (s, 4H), 2.52 (s, 4H), 2.36 (s, 3H).

*6.1.11. General procedure for the synthesis of **45a-k**, **45p**, and **45q***

A mixture of **44a/b/c/d/e/f/g/h/i/j/k/p/q** (1.3 mmol, 1.0 eq.) in 5 mL of 20% H_2SO_4 was stirred at reflux overnight. The reaction mixture was alkalified pH to 9 by saturated $NaHCO_3$ aqueous solution

and extracted with dichloromethane (30 mL). The organic layer was dried over Na_2SO_4 and concentrated to give the desired product.

*2-Methoxy-6-(4-methylpiperazin-1-yl)pyrimidine-4-carbaldehyde (**45a**)*

Compound **45a** was obtained as a yellow oil (yield 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (s, 1H), 6.74 (s, 1H), 3.98 (s, 4H), 3.72 (s, 3H), 2.46 (s, 4H), 2.32 (s, 3H).

*2-Ethoxy-6-(4-methylpiperazin-1-yl)pyrimidine-4-carbaldehyde (**45b**)*

Compound **45b** was obtained as a yellow solid (yield 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.79 (s, 1H), 6.73 (s, 1H), 4.40 (q, *J* = 7.2 Hz, 2H), 3.92-3.44 (m, 4H), 2.54-2.40 (m, 4H), 2.33 (s, 3H), 1.41 (t, *J* = 7.2 Hz, 3H). MS (ESI/APCI) m/z 282.9 $[M+MeOH+H]^+$.

*2-Isopropoxy-6-(4-methylpiperazin-1-yl)pyrimidine-4-carbaldehyde (**45c**)*

Compound **45c** was obtained as a yellow solid (yield 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.79 (s, 1H), 6.71 (s, 1H), 5.40-5.18 (m, 1H), 3.75 (s, 4H), 2.56-2.48 (m, 4H), 2.36 (s, 3H), 1.38 (d, *J* = 6.0 Hz, 6H). MS (ESI/APCI) m/z 296.9 $[M+MeOH+H]^+$.

*2-Amino-6-(4-methylpiperazin-1-yl)pyrimidine-4-carbaldehyde (**45d**)*

Compound **45d** was obtained as a yellow solid (yield 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.73 (s, 1H), 6.52 (s, 1H), 5.35 (s, 2H), 3.69 (s, 4H), 2.47 (s, 4H), 2.34 (s, 3H). MS (ESI/APCI) m/z 221.9 $[M+H]^+$.

*2-(Methylamino)-6-(4-methylpiperazin-1-yl)pyrimidine-4-carbaldehyde (**45e**)*

Compound **45e** was obtained as a yellow solid (yield 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (s, 1H), 6.45 (s, 1H), 3.69 (s, 4H), 2.98 (d, *J* = 4.8 Hz, 3H), 2.47-2.43 (m, 4H), 2.33 (s, 3H).

*2-(Ethylamino)-6-(4-methylpiperazin-1-yl)pyrimidine-4-carbaldehyde (**45f**)*

Compound **45f** was obtained as a yellow solid (yield 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.73 (s, 1H), 6.44 (s, 1H), 3.69 (s, 4H), 3.52-3.35 (m, 2H), 2.46 (t, *J* = 5.2 Hz, 4H), 2.34 (s, 3H), 1.23 (t, *J* = 7.2 Hz, 4H).

*2-(Isopropylamino)-6-(4-methylpiperazin-1-yl)pyrimidine-4-carbaldehyde(**45g**)*

Compound **45g** was obtained as a yellow solid (yield 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.71 (s, 1H), 6.42 (s, 1H), 4.93 (s, 1H), 4.22–3.96 (m, 1H), 3.72 (s, 4H), 2.53 (s, 4H), 2.38 (s, 3H), 1.23 (d, *J* = 5.6 Hz, 6H). MS (ESI/APCI) m/z 263.9 $[M+H]^+$.

*2-(Dimethylamino)-6-(4-methylpiperazin-1-yl)pyrimidine-4-carbaldehyde (**45h**)*

Compound **45h** was obtained as a yellow solid (yield 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.76 (s, 1H), 6.39 (s, 1H), 3.68 (s, 4H), 3.18 (s, 6H), 2.45 (s, 4H), 2.33 (s, 3H).

*2-((2-Hydroxyethyl)(methyl)amino)-6-(4-methylpiperazin-1-yl)pyrimidine-4-carbaldehyde (**45i**)*

Compound **45i** was obtained as a yellow solid (yield 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.73 (s, 1H), 6.42 (s, 1H), 3.92–3.84 (m, 2H), 3.84–3.76 (m, 2H), 3.70 (s, 4H), 3.22 (s, 3H), 2.61 (s, 1H), 2.49 (s, 4H), 2.35 (s, 3H). MS (ESI/APCI) m/z 311.9 $[M+MeOH+H]^+$.

*6-(4-Methylpiperazin-1-yl)-2-morpholinopyrimidine-4-carbaldehyde (**45j**)*

Compound **45j** was obtained as a yellow solid (yield 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (s, 1H), 6.44 (s, 1H), 3.83-3.78 (m, 4H), 3.76-3.74 (m, 4H), 3.67-3.65 (m, 4H), 2.45 (t, *J* = 5.0 Hz, 4H), 2.33 (s, 3H).

*6-(4-Methylpiperazin-1-yl)-2-(pyrrolidin-1-yl)pyrimidine-4-carbaldehyde (**45k**)*

Compound **45k** was obtained as a yellow solid (yield 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (s, 1H), 6.40 (s, 1H), 3.67 (s, 4H), 3.58 (s, 4H), 2.44 (s, 4H), 2.33 (s, 3H), 1.96 (s, 4H).

*6-(4-Methylpiperazin-1-yl)-2-phenylpyrimidine-4-carbaldehyde (**45p**)*

Compound **45p** was obtained as a colorless oil (yield 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.01

(s, 1H), 8.53-8.41 (m, 2H), 7.52-7.45 (m, 3H), 6.98 (s, 1H), 3.85 (s, 4H), 2.53 (d, *J* = 4.6 Hz, 4H), 2.37 (s, 3H).

*2-(1-Methyl-1H-pyrazol-4-yl)-6-(4-methylpiperazin-1-yl)pyrimidine-4-carbaldehyde (**45q**)*

Compound **45q** was obtained as a colorless oil (yield 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.92 (s, 1H), 8.14 (s, 1H), 8.08 (s, 1H), 6.86 (s, 1H), 3.97 (s, 3H), 3.94-3.74 (m, 4H), 2.68-2.49 (m, 4H), 2.41 (s, 3H).

*6.1.12. General procedure for the synthesis of **1-18***

To a solution of **45a-r** (1.1 eq.) and *N*-methyl-5,6,7,8-tetrahydroquinolin-8-amine or **52** (0.21 mmol, 1.0 eq.) in 5 mL of DCE was added AcOH (1.0 eq.). After stirred at room temperature for 10 min, $NaBH(OAc)_3$ (1.5 eq.) was added. The mixture was stirred at room temperature overnight and then quenched with saturated $NaHCO_3$ aqueous solution (10 mL). The aqueous layer was extracted with dichloromethane (10 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 100/1 to 50/1) to give the desired product.

*N-((2-Methoxy-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**1**)*

Compound **1** was obtained as a colorless oil (yield 28%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J* = 4.4 Hz, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.09-7.02 (m, 1H), 6.87 (s, 1H), 4.03 (t, *J* = 7.4 Hz, 1H), 3.88 (s, 3H), 3.76-3.66 (m, 4H), 3.58 (s, 2H), 2.86-2.75 (m, 1H), 2.74-2.65 (m, 1H), 2.44 (t, *J* = 5.2 Hz, 4H), 2.37 (s, 3H), 2.32 (s, 3H), 2.15-2.06 (m, 1H), 2.05-1.97 (m, 1H), 1.97-1.87 (m, 1H), 1.72-1.66 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.1, 165.2, 164.5, 157.5, 147.4, 136.8, 134.3, 121.8, 95.0, 62.6, 59.2, 54.9, 54.1, 46.3, 44.1, 39.4, 29.4, 23.0, 21.0. HRMS (ESI): calcd for $C_{21}H_{31}N_6O$ $[M+H]^+$ 383.2559, found 383.2553. Purity: 98.4%

*N-((2-Ethoxy-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**2**)*

Compound **2** was obtained as a colorless oil (yield 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (d, *J* = 4.0 Hz, 1H), 7.36 (d, *J* = 7.6 Hz, 1H), 7.13-7.00 (m, 1H), 6.87 (s, 1H), 4.31 (q, *J* = 7.2 Hz, 2H), 4.08-3.96 (m, 1H), 3.69 (s, 4H), 3.57 (s, 2H), 2.88-2.76 (m, 1H), 2.74-2.65 (m, 1H), 2.49-2.42 (m, 4H), 2.39 (s, 3H), 2.32 (s, 3H), 2.14-2.06 (m, 1H), 2.05-1.98 (m, 1H), 1.98-1.87 (m, 1H), 1.75-1.70 (m, 1H), 1.37 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.2, 164.8, 164.5, 157.5, 147.4, 136.8, 134.3, 121.8, 94.8, 62.5, 62.5, 59.2, 55.0, 46.3, 44.0, 39.4, 29.4, 22.9, 21.4, 14.8. MS (ESI/APCI) m/z 396.9 $[M+H]^+$. Purity: 97.9%

*N-((2-Isopropoxy-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**3**)*

Compound **3** was obtained as a colorless oil (yield 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J* = 4.8 Hz, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.08-7.02 (m, 1H), 6.86 (s, 1H), 5.25-5.12 (m, 1H), 4.10-3.99 (m, 1H), 3.75-3.64 (m, 4H), 3.56 (s, 2H), 2.85-2.76 (m, 1H), 2.74-2.65 (m, 1H), 2.44 (t, *J* = 5.0 Hz, 4H), 2.38 (s, 3H), 2.32 (s, 3H), 2.13-2.06 (m, 1H), 2.05-1.98 (m, 1H), 1.98-1.87 (m, 1H), 1.69-1.64 (m, 1H), 1.33 (d, *J* = 6.0 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.3, 164.5, 164.4, 157.6, 147.4, 136.7, 134.3, 121.7, 94.6, 69.0, 62.6, 59.3, 54.9, 46.3, 44.1, 39.4, 29.4, 22.9, 22.2, 21.3. MS (ESI/APCI) m/z 410.9 $[M+H]^+$. Purity: 96.3%

*N-((2-Amino-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**4**)*

Compound **4** was obtained as a white solid (yield 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J*

= 4.4 Hz, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.09-6.98 (m, 1H), 6.57 (s, 1H), 4.75 (s, 2H), 4.08-3.99 (m, 1H), 3.68-3.60 (m, 4H), 3.51 (s, 2H), 2.86-2.74 (m, 1H), 2.74-2.63 (m, 1H), 2.47-2.40 (m, 4H), 2.34 (s, 3H), 2.31 (s, 3H), 2.10-2.06 (m, 1H), 2.04-1.98 (m, 1H), 1.96-1.87 (m, 1H), 1.74-1.64 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.6, 163.8, 162.4, 157.6, 147.3, 136.7, 134.3, 121.7, 92.7, 62.7, 59.4, 55.0, 46.3, 43.9, 39.1, 29.4, 23.2, 21.4. HRMS (ESI): calcd for $C_{20}H_{30}N_7$ $[M+H]^+$ 368.2563, found 368.2553. Purity: 96.2%

*N-Methyl-N-((2-(methylamino)-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**5**)*

Compound **5** was obtained as a colorless oil (yield 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J* = 4.4 Hz, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.10-7.00 (m, 1H), 6.47 (s, 1H), 4.87 (s, 1H), 4.13-3.98 (m, 1H), 3.67 (m, 4H), 3.50 (s, 2H), 2.92 (d, *J* = 4.4 Hz, 3H), 2.86-2.74 (m, 1H), 2.73-2.64 (m, 1H), 2.44 (t, *J* = 5.0 Hz, 4H), 2.32 (s, 6H), 2.13-2.05 (m, 1H), 2.03-1.97 (m, 1H), 1.96-1.87 (m, 1H), 1.69-1.61 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.3, 163.7, 162.5, 157.7, 147.4, 136.7, 134.3, 121.7, 91.3, 62.7, 59.4, 55.0, 46.4, 43.9, 39.3, 29.5, 28.5, 23.3, 21.4. MS (ESI/APCI) m/z 381.9 $[M+H]^+$. Purity: 98.5%

*N-((2-(Ethylamino)-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**6**)*

Compound **6** was obtained as a colorless oil (yield 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J* = 4.4 Hz, 1H), 7.34 (d, *J* = 7.6 Hz, 1H), 7.08-7.00 (m, 1H), 6.48 (s, 1H), 4.76 (s, 1H), 4.04 (t, *J* = 7.6 Hz, 1H), 3.65 (s, 4H), 3.55-3.43 (m, 2H), 3.43-3.31 (m, 2H), 2.85-2.75 (m, 1H), 2.74-2.62 (m, 1H), 2.43 (s, 4H), 2.35 (s, 3H), 2.32 (s, 3H), 2.14-2.04 (m, 1H), 2.03-1.96 (m, 1H), 1.96-1.86 (m, 1H), 1.74-1.62 (s, 1H), 1.17 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.4, 163.7, 161.9, 157.7, 147.4, 136.7, 134.3, 121.7, 91.3, 62.7, 59.4, 55.0, 46.4, 43.9, 39.3, 36.3, 29.5, 23.3, 21.4, 15.2. MS (ESI/APCI) m/z395.9 $[M+H]^+$. Purity: 98.1%

*N-((2-(Isopropylamino)-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**7**)*

Compound **7** was obtained as a colorless oil (yield 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (d, *J* = 4.4 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.08-7.02 (m, 1H), 6.48 (s, 1H), 4.60 (s, 1H), 4.13-4.00 (m, 2H), 3.72-3.60 (m, 4H), 3.55-3.38 (m, 2H), 2.84-2.75 (m, 1H), 2.73-2.64 (m, 1H), 2.47-2.40 (m, 4H), 2.36 (s, 3H), 2.32 (s, 3H), 2.14-2.04 (s, 1H), 2.03-1.96 (m, 1H), 1.92-1.86 (m, 1H), 1.68-1.64 (m, 1H), 1.18 (d, *J* = 6.4 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.0, 163.7, 161.0, 157.7, 147.4, 136.7, 134.3, 121.7, 91.1, 62.7, 59.4, 55.0, 46.4, 44.0, 42.8, 39.3, 29.8, 29.5, 23.3, 23.1, 21.4. MS (ESI/APCI) m/z 410.0 $[M+H]^+$. Purity: 95.9%

*N-((2-(Dimethylamino)-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**8**)*

Compound **8** was obtained as a colorless oil (yield 18%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (d, *J* = 4.4 Hz, 1H), 7.34 (d, *J* = 7.2 Hz, 1H), 7.08-7.00 (m, 1H), 6.48 (s, 1H), 4.10-4.00 (m, 1H), 3.65 (s, 4H), 3.55-3.49 (m, 2H), 3.10 (s, 6H), 2.86-2.73 (m, 1H), 2.74-2.62 (m, 1H), 2.46-2.39 (m, 4H), 2.37 (s, 3H), 2.31 (s, 3H), 2.14-2.06 (m, 1H), 2.05-1.96 (m, 1H), 1.97-1.92 (m, 1H), 1.74-1.62 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.4, 163.5, 162.3, 157.7, 147.3, 136.7, 134.2, 121.6, 90.2, 62.6, 59.7, 55.0, 46.3, 43.9, 39.3, 37.0, 29.4, 23.1, 21.3. HRMS (ESI): calcd for $C_{22}H_{34}N_7$$[M+H]^+$ 396.2876, found 396.2882. Purity: 99.4%

*2-(Methyl(4-((methyl(5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)amino)ethan-1-ol (**9**)*

Compound **9** was obtained as a colorless oil (yield 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J*

= 4.8 Hz, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.10-6.98 (m, 1H), 6.49 (s, 1H), 4.10-3.98 (m, 1H), 3.83 (t, *J* = 4.6 Hz, 2H), 3.69-3.63 (m, 6H), 3.50 (d, *J* = 5.6 Hz, 2H), 3.15 (s, 3H), 2.86-2.74 (m, 1H), 2.72-2.63 (m, 1H), 2.46-2.40 (m, 4H), 2.34 (s, 3H), 2.32 (s, 3H), 2.13-2.05 (m, 1H), 2.04-1.97 (m, 1H), 1.94-1.86 (m, 1H), 1.68-1.60 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.9, 163.2, 162.5, 157.7, 147.3, 136.7, 134.3, 121.7, 91.0, 63.7, 62.7, 59.3, 54.9, 53.4, 46.3, 44.0, 39.3, 36.9, 29.4, 23.3, 21.3. MS (ESI/APCI) m/z 425.9 $[M+H]^+$. Purity: 99.8%

*N-Methyl-N-((6-(4-methylpiperazin-1-yl)-2-morpholinopyrimidin-4-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**10**)*

Compound **10** was obtained as a colorless oil (yield 29%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J* = 4.8 Hz, 1H), 7.34 (d, *J* = 7.6 Hz, 1H), 7.13-7.00 (m, 1H), 6.57 (s, 1H), 4.04 (t, *J* = 7.2 Hz, 1H), 3.72 (s, 8H), 3.68-3.60 (m, 4H), 3.55-3.47 (m, 2H), 2.87-2.76 (m, 1H), 2.74-2.63 (m, 1H), 2.43 (t, *J* = 5.2 Hz, 4H), 2.37 (s, 3H), 2.32 (s, 3H), 2.14-2.06 (m, 1H), 2.05-1.98 (m, 1H), 1.98-1.87 (m, 1H), 1.75-1.62 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.8, 163.5, 161.8, 157.8, 147.4, 136.7, 134.2, 121.7, 91.6, 67.2, 62.7, 59.6, 55.0, 46.4, 44.6, 44.0, 39.5, 29.4, 23.2, 21.3. MS (ESI/APCI) m/z 437.9 $[M+H]^+$. Purity: 97.4%

*N-Methyl-N-((6-(4-methylpiperazin-1-yl)-2-(pyrrolidin-1-yl)pyrimidin-4-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**11**)*

Compound **11** was obtained as a colorless oil (yield 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J* = 4.4 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.10-6.98 (m, 1H), 6.51 (s, 1H), 4.07 (t, *J* = 7.4 Hz, 1H), 3.70-3.63 (m, 4H), 3.57-3.48 (m, 6H), 2.87-2.74 (m, 1H), 2.73-2.62 (m, 1H), 2.43 (t, *J* = 5.0 Hz, 4H), 2.37 (s, 3H), 2.32 (s, 3H), 2.15-2.07 (m, 1H), 2.04-1.98 (m, 1H), 1.96-1.87 (m, 5H), 1.68-1.62 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.5, 163.5, 160.5, 157.8, 147.4, 136.6, 134.2, 121.6, 90.2, 62.6, 59.7, 55.1, 46.6, 46.4, 43.9, 39.3, 29.5, 25.7, 23.1, 21.4. MS (ESI/APCI) m/z 421.9 $[M+H]^+$. Purity: 98.0%

*N-Methyl-N-((2-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**12**)*

Compound **12** was obtained as a colorless oil (yield 39%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J* = 3.6 Hz, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.08-7.01 (m, 2H), 4.04 (t, *J* = 7.4 Hz, 1H), 3.76-3.67 (m, 4H), 3.60 (s, 2H), 2.86-2.74 (m, 1H), 2.74-2.65 (m, 1H), 2.49-2.43 (m, 7H), 2.38 (s, 3H), 2.33 (s, 3H), 2.15-2.07 (m, 1H), 2.06-1.98 (m, 1H), 1.98-1.85 (m, 1H), 1.74-1.62 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.8, 166.6, 162.9, 157.5, 147.4, 136.7, 134.3, 121.8, 97.9, 62.7, 59.1, 54.9, 46.3, 43.8, 39.4, 29.4, 26.1, 23.1, 21.4. HRMS (ESI): calcd for $C_{21}H_{31}N_6$ $[M+H]^+$ 367.2610, found 367.2604. Purity: 99.1%

*N-((2-Ethyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**13**)*

Compound **13** was obtained as a yellow oil (yield 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (d, *J* = 4.4 Hz, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.08-7.01 (m, 2H), 4.04 (t, *J* = 7.4 Hz, 1H), 3.72 (s, 4H), 3.61 (s, 2H), 2.85-2.76 (m, 1H), 2.73-2.68 (m, 3H), 2.46 (t, *J* = 5.0 Hz, 4H), 2.39 (s, 3H), 2.33 (s, 3H), 2.18-2.06 (m, 1H), 2.05-1.98 (m, 1H), 1.97-1.90 (m, 1H), 1.75-1.70 (m, 1H), 1.28-1.25 (m, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.7, 167.8, 163.0, 157.6, 147.4, 136.7, 134.3, 121.7, 98.0, 62.7, 59.1, 55.0, 46.4, 43.8, 39.4, 32.7, 29.5, 23.1, 21.4, 12.8. MS (ESI/APCI) m/z 380.9 $[M+H]^+$. Purity: 95.0%

*N-((2-Isopropyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**14**)*

Compound **14** was obtained as a colorless oil (yield 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (d,

J = 4.4Hz, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.07-7.00 (m, 2H), 4.04 (t, *J* = 7.4 Hz, 1H), 3.78-3.68 (m, 4H), 3.61 (s, 2H), 2.98-2.87(m, 1H), 2.85-2.75 (m, 1H), 2.72-2.65 (m, 1H), 2.52-2.43 (m, 4H), 2.40 (s, 3H), 2.33 (s, 3H), 2.18-2.08 (m, 1H), 2.06-1.98 (m, 1H), 1.97-1.86 (m, 1H), 1.72-1.65 (m, 1H), 1.28-1.21 (m, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.9, 167.8, 163.0, 157.6, 147.4, 136.7, 134.3, 121.7, 98.1, 62.8, 59.1, 55.0, 46.4, 43.8, 39.5, 37.6, 29.5, 23.1, 22.0, 21.4. MS (ESI/APCI) m/z 394.9 $[M+H]^+$. Purity: 95.1%

*N-((2-Cyclopropyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**15**)*

Compound **15** was obtained as a colorless oil (yield 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J* = 4.8 Hz, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.08-6.98 (m, 2H), 4.03 (t, *J* = 7.4 Hz, 1H), 3.70-3.64 (m, 4H), 3.60 (s, 2H), 2.86-2.75 (m, 1H), 2.74-2.64 (m, 1H), 2.46-2.40 (m, 4H), 2.39 (s, 3H), 2.32 (s, 3H), 2.15-2.06 (m, 2H), 2.03-1.99 (m, 1H), 1.95-1.86 (m, 1H), 1.74-1.64 (m, 1H), 1.04-1.00 (m, 2H), 0.91-0.84 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.3, 167.4, 162.8, 157.6, 147.4, 136.7, 134.3, 121.7, 97.9, 62.6, 59.1, 54.9, 46.3, 43.7, 39.3, 29.4, 23.0, 21.4, 18.0, 9.7. HRMS (ESI): calcd for $C_{23}H_{33}N_6$ $[M+H]^+$ 393.2767, found 393.2760. Purity: 97.5%

*N-Methyl-N-((6-(4-methylpiperazin-1-yl)-2-phenylpyrimidin-4-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**16**)*

Compound **16** was obtained as a colorless oil (yield 20%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (s, 1H), 8.39-8.29 (m, 2H), 7.46-7.38 (m, 3H), 7.36 (d, *J* = 7.6 Hz, 1H), 7.18 (s, 1H), 7.12-7.01 (m, 1H), 4.17-4.02 (m, 1H), 3.82 (s, 4H), 3.74 (s, 2H), 2.90-2.76 (m, 1H), 2.75-2.64 (m, 1H), 2.55-2.47 (m, 4H), 2.45 (s, 3H), 2.35 (s, 3H), 2.22-2.10 (m, 1H), 2.09-2.01 (m, 1H), 2.01-1.92 (m, 1H), 1.78-1.64 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.4, 163.3, 163.1, 157.6, 147.5, 139.0, 136.8, 134.3, 130.0, 128.3, 128.2, 121.8, 99.1, 62.7, 59.4, 55.0, 46.4, 44.0, 39.5, 29.5, 23.1, 21.4. HRMS (ESI): calcd for $C_{26}H_{33}N_6$$[M+H]^+$ 429.2767, found 429.2760. Purity: 96.1%

*N-Methyl-N-((2-(1-methyl-1H-pyrazol-4-yl)-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**17**)*

Compound **17** was obtained as a colorless oil (yield 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, *J* = 4.8 Hz, 1H), 8.07 (s, 1H), 8.00 (s, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.09-7.02 (m, 2H), 4.12-4.02 (m, 1H), 3.92 (s, 3H), 3.78-3.72 (m, 4H), 3.66 (s, 2H), 2.86-2.76 (m, 1H), 2.75-2.65 (m, 1H), 2.52-2.45 (m, 4H), 2.41 (s, 3H), 2.34 (s, 3H), 2.15-2.08 (m, 1H), 2.09-2.03 (m, 1H), 1.98-1.88 (m, 1H), 1.75-1.65 (m, 1H)). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.2, 162.9, 159.8, 157.6, 147.4, 139.8, 136.8, 134.3, 131.0, 124.2, 121.8, 98.2, 62.7, 59.3, 55.0, 46.4, 43.9, 39.4, 39.2, 29.5, 23.1, 21.4. HRMS (ESI): calcd for $C_{24}H_{33}N_8$ $[M+H]^+$ 433.2828, found 433.2819. Purity: 99.5%

*(S)-N-Methyl-N-((6-(4-methylpiperazin-1-yl)-2-(trifluoromethyl)pyrimidin-4-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**18**)*

Compound **18** was obtained as a colorless oil (yield 15%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (d, *J* = 3.6 Hz, 1H), 7.46 (s, 1H), 7.36 (d, *J* = 7.6 Hz, 1H), 7.08-7.04 (m, 1H), 4.06-3.96 (m, 1H), 3.84-3.72 (m, 4H), 3.72-3.60 (m, 2H), 2.87-2.75 (m, 1H), 2.74-2.61 (m, 1H), 2.53-2.42 (m, 4H), 2.42 (s, 3H), 2.34 (s, 3H), 2.16-2.08 (m, 1H), 2.07-1.96 (m, 1H), 1.96-1.86 (m, 1H), 1.76-1.64 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.8, 162.6, 157.1, 155.5 (q, *J* = 34.5 Hz), 147.3, 136.8, 134.3, 121.8, 119.7 (q, *J* = 283.5 Hz), 102.0, 62.5, 58.5, 54.6, 46.0, 43.8, 39.3, 29.3, 22.9, 21.2. HRMS (ESI): calcd for $C_{21}H_{28}F_3N_6$ $[M+H]^+$ 421.2328, found 421.2312. $[\alpha]_D^{25}$= -30 (c = 0.2, $CHCl_3$). Purity: 98.1%

*6.1.13. 6-(Chloromethyl)-2-methylpyrimidin-4-ol (**47**)*

To a mixture of **46** (22 g, 136 mmol) and acetamidine hydrochloride (17 g, 150 mmol) in 180 mL

of EtOH was added DBU (41 g, 272 mmol) at 0°C. The mixture was stirred at room temperature overnight and then concentrated. The residue was acidified with 2 N HCl to pH = 4 and extracted with ethyl acetate (200 mL x 6). The combined organic layer was dried over Na_2SO_4 and concentrated. The crude material was purified by trituration with ethyl acetate to give the desired compound (8 g, 37%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.49 (br s, 1H), 6.30 (s, 1H), 4.44 (s, 2H), 2.28 (s, 3H). MS (ESI/APCI) m/z 158.9 $[M+H]^+$.

*6.1.14. 4-Chloro-6-(chloromethyl)-2-methylpyrimidine (**48**)*

A mixture of compound **47** (8.0 g, 50 mmol) and $POCl_3$ (40 mL) was stirred at reflux for 2 h. The resulting solution was evaporated and ethyl acetate (200 mL) was added. The mixture was poured into iced water, and the organic layer was separated. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (petroleum ether /ethyl acetate = 50/1) to give the desired product (5.2 g, 59%) as a slight yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (s, 1H), 4.55 (s, 2H), 2.71 (s, 3H). MS (ESI/APCI) m/z 176.9 $[M+H]^+$.

*6.1.15. General procedure for the synthesis of **50a** and **50b***

A solution of **49** (3.0 mmol, 1.0 eq.) and corresponding amine (10.0 eq.) in 10 mL of THF was stirred at 80°C overnight in a sealed tube. The reaction mixture was quenched with saturated $NaHCO_3$ aqueous solution (10 mL) and extracted with dichloromethane (10 mL x 3). The combined organic layer was dried over Na_2SO_4 and concentrated to give the desired product, which was used directly in the next step without further purification.

*N-Ethyl-5,6,7,8-tetrahydroquinolin-8-amine (**50a**)*

Compound **50a** was obtained as a brown oil (960 mg, crude). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.37 (d, *J* =7.2 Hz, 1H), 7.11-7.01 (m, 1H), 3.86-3.74 (m, 1H), 2.88-2.70 (m, 4H), 2.19-2.14 (m, 1H), 2.05-1.95 (m, 2H), 1.78-1.71 (m, 2H), 1.21-1.18 (m, 3H). MS (ESI/APCI) m/z 177.0 $[M+H]^+$.

*N-(Cyclopropylmethyl)-5,6,7,8-tetrahydroquinolin-8-amine (**50b**)*

Compound **50b** was obtained as a brown oil (240 mg, crude). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.37 (d, *J* =7.2 Hz, 1H), 7.11-7.01 (m, 1H), 3.86-3.74 (m, 1H), 2.88-2.70 (m, 4H), 2.19-2.14 (m, 1H), 2.05-1.95 (m, 2H), 1.78-1.71 (m, 2H), 1.21-1.18 (m, 3H). MS (ESI/APCI) m/z 203.0 $[M+H]^+$.

*6.1.16. General procedure for the synthesis of **51a** and **51b***

A mixture of **50a** or **50b** (0.28 mmol, 1.0 eq.), **48** (1.1 eq.), KI (0.01 eq.) and DIPEA (2.5 eq.) in 1.5 mL of MeCN was stirred at room temperature overnight. The reaction solution was evaporated to remove most of MeCN, diluted with saturated aqueous $NaHCO_3$ solution (10 mL) and extracted with dichloromethane (10 mL x 3). The combined organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 200/1) to give the desired product.

*N-((6-Chloro-2-methylpyrimidin-4-yl)methyl)-N-ethyl-5,6,7,8-tetrahydroquinolin-8-amine (**51a**)*

Compound **51a** was obtained as a slight yellow oil (yield 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, *J* = 4.4 Hz, 1H), 7.80 (s, 1H), 7.32 (d, *J* = 7.6 Hz, 1H), 7.06-6.98 (m, 1H), 4.18-4.07 (m, 1H), 3.81 (d, *J* = 17.6 Hz, 1H), 3.65 (d, *J* = 17.6 Hz, 1H), 2.93-2.70 (m, 4H), 2.62 (s, 3H), 2.17-2.13 (m, 1H), 2.05-1.99 (m, 1H), 1.82-1.76 (m, 1H), 1.72-1.66 (m, 1H), 1.06 (t, *J* = 7.2 Hz, 3H).

*N-((6-Chloro-2-methylpyrimidin-4-yl)methyl)-N-(cyclopropylmethyl)-5,6,7,8-tetrahydroquinolin-8-amine (**51b**)*

Compound **51b** was obtained as a yellow oil (yield 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, *J* = 4.4 Hz, 1H), 7.91 (s, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.06-6.98 (m, 1H), 4.32-4.18 (m, 1H), 3.95-3.80 (m, 1H), 3.80-3.60 (m, 1H), 2.75-2.66 (m, 4H), 2.63 (s, 3H), 2.25-2.13 (m, 1H), 2.05-1.96 (m, 1H),

1.87-1.75 (m, 2H), 0.88-0.76 (m, 1H), 0.42-0.30 (m, 2H), 0.12-0.06 (m, 1H).

*6.1.17. General procedure for the synthesis of **19** and **20***

To a solution of **51a** or **51b** (0.12 mmol, 1.0 eq.) in EtOH (1 mL) was added Et_3N (2.0 eq.) and *N*-methyl piperazine (1.2 eq.). The reaction was stirred at 80°C overnight, and then quenched with saturated $NaHCO_3$ aqueous solution (20 mL). The aqueous layer was extracted with dichloromethane (20 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 200/1) to give the desired product.

*N-Ethyl-N-((2-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**19**)*

Compound **19** was obtained as a colorless oil (yield 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.38-7.29 (m, 1H), 7.21 (s, 1H), 7.03 (s, 1H), 4.13 (s, 1H), 3.73 (s, 4H), 3.64-3.45 (m, 2H), 2.90-2.60 (m, 4H), 2.55-2.40 (m, 7H), 2.34 (s, 3H), 2.20-2.06 (m, 1H), 2.06-1.90 (m, 1H), 1.90-1.78 (m, 1H), 1.78-1.70 (m, 1H), 1.10-0.98 (m, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.8, 166.4, 163.0, 158.3, 147.3, 136.5, 134.4, 121.6, 98.0, 61.1, 56.7, 55.0, 47.4, 46.4, 43.9, 29.5, 26.1, 25.9, 21.6, 14.6. MS (ESI/APCI) m/z 380.9 $[M+H]^+$. Purity: 99.4%

*N-(Cyclopropylmethyl)-N-((2-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**20**)*

Compound **20** was obtained as a yellow oil (yield 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.32 (d, *J* = 7.2 Hz, 2H), 7.02 (s, 1H), 4.30-4.18 (m, 1H), 3.80-3.55 (m, 6H), 2.85-2.52 (m, 4H), 2.52-2.40 (m, 7H), 2.35 (s, 3H), 2.20-2.10 (m, 1H), 2.02-1.90 (m, 1H), 1.88-1.76 (m, 2H), 0.90-0.78 (m, 1H), 0.45-0.30 (m, 2H), 0.12-0.03 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.8, 166.3, 163.0, 158.3, 147.2, 136.5, 134.3, 121.5, 98.0, 61.5, 58.1, 56.7, 54.9, 46.3, 43.8, 29.5, 26.1, 26.1, 21.7, 10.8, 4.5, 3.8. HRMS (ESI): calcd for $C_{24}H_{35}N_6$ $[M+H]^+$ 407.2923, found 407.2912. Purity: 98.0%

*6.1.18. (S)-N-((6-Chloro-2-methylpyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**53**)*

A mixture of **52** (90 mg, 0.56 mmol), **48** (108 mg, 0.61 mmol), KI (10 mg, 0.06 mmol) and DIPEA (180 mg, 1.4 mmol) in 10 mL of MeCN was stirred at room temperature overnight. The reaction solution was evaporated to remove most of MeCN, diluted with saturated aqueous $NaHCO_3$ solution and extracted with dichloromethane. The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 100/1 to 50/1) to give the desired product (110 mg, 65%) as a slight yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.74-7.66 (m, 1H), 7.40-7.32 (m, 1H), 7.11-7.02 (m, 1H), 4.05-3.95 (m, 1H), 3.72 (s, 2H), 2.86-2.68 (m, 2H), 2.63 (s, 3H), 2.40 (s, 3H), 2.18-2.09 (m, 1H), 2.07-1.98 (m, 1H), 1.91-1.82 (m, 1H), 1.76-1.64 (m, 1H). $[\alpha]_D^{25}$= -29 (c = 0.2, $CHCl_3$). MS (ESI/APCI) m/z 302.8 $[M+H]^+$.

*6.1.19. General procedure for the synthesis of **23-26**, **29-30**, **32-38***

To a solution of **53** (0.1 mmol, 1.0 eq.) in EtOH (2 mL) was added Et_3N (10.0 eq.) and corresponding amine (5.0 eq.). The reaction was stirred at 85 °C overnight, and then quenched with saturated $NaHCO_3$ aqueous solution. The aqueous layer was extracted with dichloromethane. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 100/1) to give the desired product.

*(S)-N-Methyl-N-((2-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**23**)*

Compound **23** was obtained as a yellow oil (yield 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (d, *J* = 4.4 Hz, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.09-7.01 (m, 2H), 4.04 (t, *J* = 7.6 Hz, 1H), 3.71 (s, 4H), 3.60

(s, 2H), 2.88-2.75 (m, 1H), 2.74-2.65 (m, 1H), 2.48-2.44 (m, 7H), 2.39 (s, 3H), 2.33 (s, 3H), 2.16-2.06 (m, 1H), 2.04-1.98(m, 1H), 1.95-1.84 (m, 1H), 1.77-1.68 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.7, 166.6, 162.9, 157.5, 147.4, 136.8, 134.4, 121.8, 98.0, 62.7, 59.0, 54.9, 46.3, 43.8, 39.3, 29.5, 26.1, 22.9, 21.4. HRMS (ESI): calcd for $C_{21}H_{31}N_6$ $[M+H]^+$ 367.2610, found 367.2605. $[\alpha]_D^{25}$= -29 (c = 0.2, $CHCl_3$). Purity: 99.3%

*(S)-N-((6-(4-Ethylpiperazin-1-yl)-2-methylpyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**24**)*

Compound **24** was obtained as a yellow oil (yield 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54-8.46 (m, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.10-7.02 (m, 2H), 4.10-4.00 (m, 1H), 3.72 (s, 4H), 3.60 (s, 2H), 2.87-2.76 (m, 1H), 2.75-2.64 (m, 1H), 2.53-2.42 (m, 9H), 2.40 (s, 3H), 2.16-2.07 (m, 1H), 2.05-1.98 (m, 1H), 1.96-1.89 (m, 1H), 1.76-1.66 (m, 1H), 1.12 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.7, 166.6, 162.9, 157.5, 147.4, 136.8, 134.4, 121.8, 97.9, 62.7, 59.1, 52.7, 52.5, 43.9, 39.4, 29.5, 26.1, 23.1, 21.4, 12.1. HRMS (ESI): calcd for $C_{22}H_{33}N_6$ $[M+H]^+$ 381.2767, found 381.2766. $[\alpha]_D^{25}$= -24 (c = 0.2, $CHCl_3$). Purity: 99.4%

*(S)-N-((6-(4-Cyclopropylpiperazin-1-yl)-2-methylpyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**25**)*

Compound **25** was obtained as a yellow oil (yield 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (d, *J* = 3.6 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 7.07-7.03 (m, 2H), 4.08-4.01 (m, 1H), 3.70-3.63 (m, 4H), 3.61 (s, 2H), 2.83-2.74 (m, 1H), 2.73-2.69 (m, 1H), 2.68-2.63 (m, 4H), 2.47 (s, 3H), 2.40 (s, 3H), 2.16-2.07 (m, 1H), 2.05-1.96 (m, 1H), 1.95-1.86 (m, 1H), 1.69-1.66 (m, 1H), 1.65-1.59 (m, 1H), 0.51-0.43 (m, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.5, 166.5, 162.9, 157.5, 147.4, 136.8, 134.4, 121.8, 98.0, 62.6, 59.0, 53.2, 43.9, 39.3, 38.6, 29.5, 26.1, 22.9, 21.4, 6.0. MS (ESI/APCI) m/z 392.9 $[M+H]^+$. $[\alpha]_D^{25}$= -11 (c = 0.2, $CHCl_3$). Purity: 95.1%

*(S)-N-Methyl-N-((2-methyl-6-(4-(oxetan-3-yl)piperazin-1-yl)pyrimidin-4-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**26**)*

Compound **26** was obtained as a yellow solid (yield 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (d, *J* = 4.0 Hz, 1H), 7.36 (d, *J* = 7.2 Hz, 1H), 7.10-7.01 (m, 2H), 4.71-4.60 (m, 4H), 4.10-3.95 (m, 1H), 3.74 (s, 4H), 3.61 (s, 2H), 3.55-3.46 (m, 1H), 2.86-2.75 (m, 1H), 2.71-2.67 (m, 1H), 2.47 (s, 3H), 2.38 (s, 7H), 2.20-2.07 (m, 1H), 2.06-1.96 (m, 1H), 1.93-1.88 (m, 1H), 1.78-1.65 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.7, 166.6, 162.8, 157.4, 147.4, 136.8, 134.4, 121.8, 98.0, 75.5, 62.6, 59.3, 58.9, 49.5, 43.6, 39.2, 29.4, 26.1, 22.8, 21.4. MS (ESI/APCI) m/z 408.9 $[M+H]^+$. $[\alpha]_D^{25}$= -21 (c = 0.2, $CHCl_3$). Purity: 98.7%

*(S)-N-((6-((R)-3,4-Dimethylpiperazin-1-yl)-2-methylpyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**29**)*

Compound **29** was obtained as a slight yellow solid (yield 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52-8.47 (m, 1H), 7.36 (d, *J* = 7.6 Hz, 1H), 7.09-7.00 (m, 2H), 4.35 (s, 1H), 4.25 (s, 1H), 4.04 (t, *J* = 7.6 Hz, 1H), 3.68-3.55 (m, 2H), 3.14-3.03 (m, 1H), 2.88-2.82 (m, 1H), 2.82-2.76 (m, 1H), 2.74-2.63 (m, 2H), 2.46 (s, 3H), 2.37 (s, 3H), 2.31 (s, 3H), 2.29-2.18 (m, 1H), 2.14-2.06 (m, 2H), 2.04-1.97 (m, 1H), 1.93-1.87 (m, 1H), 1.73-1.64 (m, 1H), 1.17-1.09 (m, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.7, 166.6, 162.7, 157.6, 147.5, 136.8, 134.4, 121.8, 97.9 (*J* = 3.0 Hz), 62.5, 59.1, 57.7 (*J* = 6.0 Hz), 55.3 (*J* = 7.5 Hz), 50.3, 44.0, 42.7, 39.3, 29.5, 26.1, 22.9, 21.5, 17.0. HRMS (ESI): calcd for $C_{22}H_{33}N_6$ $[M+H]^+$ 381.2767, found 381.2763. $[\alpha]_D^{25}$= -6 (c = 0.2, $CHCl_3$). Purity: 90.1%

(S)-N-((6-((S)-3,4-Dimethylpiperazin-1-yl)-2-methylpyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydr

*oquinolin-8-amine (**30**)*

Compound **30** was obtained as a colorless oil (yield 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, *J* = 4.4 Hz, 1H), 7.36 (d, *J* = 7.6 Hz, 1H), 7.09-7.01 (m, 2H), 4.35 (s, 1H), 4.25 (s, 1H), 4.05 (t, *J* = 7.6 Hz, 1H), 3.68-3.55 (m, 2H), 3.14-3.02 (m, 1H), 2.89-2.82 (m, 1H), 2.80-2.74 (m, 1H), 2.74-2.63 (m, 2H), 2.46 (s, 3H), 2.37 (s, 3H), 2.31 (s, 3H), 2.28-2.19 (m, 1H), 2.14-2.06 (m, 2H), 2.04-1.98 (m, 1H), 1.97-1.87 (m, 1H), 1.73-1.64 (m, 1H), 1.16-1.10 (m, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.6, 166.6, 162.8, 157.5, 147.4, 136.8, 134.2, 121.8, 97.9 (*J* = 4.5 Hz), 62.5, 59.0, 57.7 (*J* = 4.5 Hz), 55.3 (*J* = 6.0 Hz), 50.3, 44.0, 42.7, 39.3, 29.5, 26.1, 22.8, 21.4, 17.0. MS (ESI/APCI) m/z 380.9 $[M+H]^+$. $[\alpha]_D^{25}$= -22 (c = 0.2, $CHCl_3$). Purity: 96.6%

*(S)-N-((6-((R)-Hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl)-2-methylpyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**32**)*

Compound **32** was obtained as a yellow oil (yield 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55-8.48 (m, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.14-7.02 (m, 2H), 4.75-4.35 (m, 2H), 4.11-3.97 (m, 1H), 3.61 (s, 2H), 3.15-3.08 (m, 2H), 3.05-2.96 (m, 1H), 2.86-2.76 (m, 1H), 2.74-2.57 (m, 2H), 2.46 (s, 3H), 2.38 (s, 3H), 2.23-1.83 (m, 9H), 1.74-1.63 (m, 1H), 1.55-1.42 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.5, 166.5, 163.0, 157.5, 147.5 (*J* = 3.0 Hz), 136.8, 134.4, 121.8, 98.2, 62.6 (*J* = 4.5 Hz), 62.4 (*J* = 6.0 Hz), 59.0, 53.7, 51.6 (*J* = 7.5 Hz), 48.7, 43.4, 39.3, 29.5, 27.5, 26.1, 22.9, 21.4 (*J* = 9.0 Hz). MS (ESI/APCI) m/z 392.9 $[M+H]^+$. $[\alpha]_D^{25}$= -9 (c = 0.2, $CHCl_3$). Purity: 99.0%

*(S)-N-((6-((S)-Hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl)-2-methylpyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**33**)*

Compound **33** was obtained as a yellow oil (yield 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.36 (d, *J* = 7.6 Hz, 1H), 7.11-7.03 (m, 2H), 4.68-4.37 (m, 2H), 4.09-3.99 (m, 1H), 3.68-3.55 (m, 2H), 3.17-3.07 (m, 2H), 3.06-2.97 (m, 1H), 2.84-2.74 (m, 1H), 2.71-2.60 (m, 2H), 2.47 (s, 3H), 2.38 (s, 3H), 2.28-2.06 (m, 3H), 2.04-1.82 (m, 6H), 1.71-1.59 (m, 1H), 1.54-1.43 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.3, 166.4, 163.0, 157.5, 147.5 (*J* = 3.0 Hz), 136.8, 134.4, 121.8, 98.2, 62.6 (*J* = 4.5 Hz), 62.5 (*J* = 6.0 Hz), 59.0, 53.7, 51.6 (*J* = 7.5 Hz), 48.7, 43.4, 39.3, 29.8, 29.5, 27.6, 26.1, 22.9, 21.4 (*J* = 9.0 Hz). MS (ESI/APCI) m/z 392.9 $[M+H]^+$. $[\alpha]_D^{25}$= -5 (c = 0.2, $CHCl_3$). Purity: 97.3%

*(S)-N-Methyl-N-((2-methyl-6-(4-morpholinopiperidin-1-yl)pyrimidin-4-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**34**)*

Compound **34** was obtained as a yellow oil (yield 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51-8.47 (m, 1H), 7.36 (d, *J* = 7.6 Hz, 1H), 7.11-7.02 (m, 2H), 4.58 (d, *J* = 12.0 Hz, 2H), 4.03 (t, *J* = 7.2 Hz, 1H), 3.72 (s, 4H), 3.61 (s, 2H), 2.88-2.76 (m, 3H), 2.74-2.65 (m, 1H), 2.56 (s, 4H), 2.46 (s, 4H), 2.38 (s, 3H), 2.15-1.96 (m, 2H), 1.95-1.85 (m, 3H), 1.76-1.65 (m, 1H), 1.55-1.42 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.5, 166.6, 162.7, 157.5, 147.4, 136.8, 134.4, 121.8, 97.9, 67.4, 62.6, 62.4, 59.0, 49.9, 43.5, 39.4, 29.5, 28.1 (*J* = 6.0 Hz), 26.1, 23.0, 21.4. MS (ESI/APCI) m/z 436.9 $[M+H]^+$. $[\alpha]_D^{25}$= -11 (c = 0.2, $CHCl_3$). Purity: 96.5%

*(S)-N-Methyl-N-((2-methyl-6-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)pyrimidin-4-yl)methyl)-5,6,7,8-Tetrahydroquinolin-8-amine (**35**)*

Compound **35** was obtained as a yellow oil (yield 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 7.41-7.31 (m, 1H), 7.13-6.92 (m, 2H), 4.63-4.50 (m, 2H), 4.08-3.92 (m, 1H), 3.59 (s, 2H), 2.87-2.75 (m, 3H), 2.74-2.55 (m, 4H), 2.54-2.40 (m, 6H), 2.37 (s, 3H), 2.28 (s, 3H), 2.16-2.07 (m, 4H), 2.02-1.85 (m, 4H), 1.73-1.62 (m, 1H), 1.50-1.37 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.7, 166.7, 162.7, 157.6, 147.5, 136.7, 134.3, 121.7, 97.9, 62.6, 62.2, 59.1, 55.5, 49.1, 46.1, 43.6, 39.4, 29.5, 28.2 (*J* = 6.0 Hz), 26.2, 23.1, 21.4. MS (ESI/APCI) m/z 449.9 $[M+H]^+$. $[\alpha]_D^{25}$= -29 (c = 0.2, $CHCl_3$). Purity:

95.9%

*(S)-N-Methyl-N-((2-methyl-6-((2-morpholinoethyl)amino)pyrimidin-4-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**36**)*

Compound **36** was obtained as a yellow oil (yield 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53-8.48 (m, 1H), 7.36 (d, *J* = 7.6 Hz, 1H), 7.10-7.02 (m, 1H), 6.75 (s, 1H), 5.52 (br s, 1H), 4.08-4.02 (m, 1H), 3.72 (s, 4H), 3.56 (s, 2H), 3.42 (s, 2H), 2.84-2.77 (m, 1H), 2.74-2.65 (m, 1H), 2.62-2.56 (m, 2H), 2.46 (s, 7H), 2.38 (s, 3H), 2.15-2.08 (m, 1H), 2.06-1.98 (m, 1H), 1.96-1.85 (m, 1H), 1.80-1.75 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ167.0, 166.4, 163.0, 157.2, 147.2, 136.6, 134.2, 121.6, 98.5, 86.9, 83.1, 58.5, 57.0, 53.2, 39.0, 37.3, 29.3, 25.7, 23.0, 21.2. MS (ESI/APCI) m/z 396.9 $[M+H]^+$. $[\alpha]_D^{25}$= -26 (c = 0.2, $CHCl_3$). Purity: 96.3%

*(S)-N-Methyl-N-((2-methyl-6-((3-morpholinopropyl)amino)pyrimidin-4-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**37**)*

Compound **37** was obtained as a yellow oil (yield 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (s, 1H), 7.37 (d, *J* = 6.8 Hz, 1H), 7.12-7.02 (m, 1H), 6.73 (s, 1H), 5.50 (s, 1H), 4.13-3.95 (m, 1H), 3.72 (s, 4H), 3.59 (s, 2H), 3.44 (s, 2H), 2.88-2.76 (m, 1H), 2.75-2.63 (m, 1H), 2.50-2.40 (m, 9H), 2.38 (s, 3H), 2.18-2.08 (m, 1H), 2.06-1.96 (m, 1H), 1.96-1.88 (m, 2H), 1.76-1.73 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ167.3, 166.6, 163.2, 157.3, 147.2, 136.5, 134.1, 121.5, 97.9, 66.9, 62.9, 58.9, 56.9, 53.7, 40.2, 39.1, 29.2, 25.8, 25.6, 23.3, 21.2. MS (ESI/APCI) m/z 410.9 $[M+H]^+$. $[\alpha]_D^{25}$= -13 (c = 0.2, $CHCl_3$). Purity: 96.2%

*(S)-2-(Methyl(2-methyl-6-((methyl(5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)pyrimidin-4-yl)amino)ethan-1-ol (**38**)*

Compound **38** was obtained as a yellow oil (yield 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50-8.45 (m, 1H), 7.41-7.32 (m, 1H), 7.10-7.00 (m, 1H), 7.00 (s, 1H), 4.10-3.96 (m, 1H), 3.88-3.80 (m, 2H), 3.78-3.68 (m, 2H), 3.62 (s, 2H), 3.14 (s, 3H), 2.86-2.74 (m, 1H), 2.75-2.63 (m, 1H), 2.46 (s, 3H), 2.38 (s, 3H), 2.16-2.06 (m, 1H), 2.06-1.98 (m, 1H), 1.96-1.85 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.5, 165.9, 163.5, 157.4, 147.4, 136.9, 134.4, 121.8, 98.3, 62.7, 59.0, 53.6, 39.3, 37.3, 29.4, 25.8, 23.1, 21.4. MS (ESI/APCI) m/z 341.9 $[M+H]^+$. $[\alpha]_D^{25}$= -44 (c = 0.2, $CHCl_3$). Purity: 97.8%

*6.1.20. General procedure for the synthesis of **27** and **28***

To a solution of **53** (0.1 mmol, 1.0 eq.), Et_3N (10.0 eq.) and corresponding amine (5.0 eq.) in 2 mL of EtOH was stirred at 80°C overnight. The reaction mixture was quenched with saturated $NaHCO_3$ aqueous solution (20 mL) and extracted with dichloromethane (20 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was diluted with ethyl acetate (2 mL) and added HCl/ ethyl acetate (3 M, 3 mL). The mixture was stirred at room temperature overnight and quenched with saturated $NaHCO_3$ aqueous solution (50 mL). The aqueous layer was extracted with ethyl acetate (30 mL x 3). The organic layers were dried over Na_2SO_4 and concentrated. The resulting residue was diluted with DCE (4 mL) and HCHO/H_2O (37 percent, 5.0 eq.) was added, followed by $NaBH(OAc)_3$ (5.0 eq.). The mixture was stirred at room temperature overnight and then quenched with saturated $NaHCO_3$ aqueous solution. The aqueous layer was extracted with dichloromethane (30 mL x 3). The combined organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 100/1) to give the desired product.

*(S)-N-((6-((R)-2,4-Dimethylpiperazin-1-yl)-2-methylpyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**27**)*

Compound **27** was obtained as a colorless oil (yield 25%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.09-6.92 (m, 2H), 4.62 (s, 1H), 4.35-4.28 (m, 1H), 4.09-3.98 (m, 1H),

3.74-3.49 (m, 2H), 3.23-3.08 (m, 1H), 2.91-2.83 (m, 1H), 2.82-2.75 (m, 1H), 2.74-2.65 (m, 2H), 2.46 (s, 3H), 2.42-2.36 (m, 3H), 2.28 (s, 3H), 2.22-2.17 (m, 1H), 2.14-2.07 (m, 1H), 2.05-1.91 (m, 3H), 1.74-1.63 (m, 1H), 1.29-1.23 (m, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.9 (d, *J* = 16.5 Hz), 166.2 (d, *J* = 9.0 Hz), 162.2 (d, *J* = 10.5 Hz), 157.2, 147.1, 136.7, 134.2 (d, *J* = 7.5 Hz), 121.6 (d, *J* = 3.0 Hz), 97.9 (d, *J* = 7.5 Hz), 62.6 (d, *J* = 90.0 Hz), 60.0 (d, *J* = 4.5 Hz), 58.7 (d, *J* = 16.5 Hz), 55.2 (d, *J* = 6.0 Hz), 46.6, 46.5, 38.9 (d, *J* = 46.5 Hz), 38.7, 29.2, 25.8, 22.7 (d, *J* = 51.0 Hz), 21.2 (d, J = 9.0 Hz), 14.7 (d, *J* = 6.0 Hz). MS (ESI/APCI) m/z 380.9 $[M+H]^+$. $[\alpha]_D^{25}$= -77 (c = 0.2, $CHCl_3$). Purity: 96.4%

*(S)-N-((6-((S)-2,4-Dimethylpiperazin-1-yl)-2-methylpyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**28**)*

Compound **28** was obtained as a colorless oil (yield 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51-8.44 (m, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.09-7.02 (m, 1H), 7.02-6.89 (m, 1H), 4.61 (s, 1H), 4.31 (d, *J* = 13.2 Hz, 1H), 4.05 (q, *J* = 7.8 Hz, 1H), 3.63 (d, *J* = 8.0 Hz, 1H), 3.56 (s, 1H), 3.21-3.10 (m, 1H), 2.89-2.83 (m, 1H), 2.81-2.74 (m, 1H), 2.74-2.67 (m, 2H), 2.46 (s, 3H), 2.37 (d, *J* = 6.0 Hz, 3H), 2.23 (s, 3H), 2.21-2.15 (m, 1H), 2.15-2.05 (m, 1H), 2.04-1.95 (m, 2H), 1.95-1.92 (m, 1H), 1.74-1.63 (m, 1H), 1.30-1.25 (m, 2H), 1.18 (d, *J* = 6.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.3 (d, *J* = 19.5 Hz), 166.4 (d, *J* = 9.0 Hz), 162.2 (d, *J* = 10.5 Hz), 157.3, 147.2, 136.6, 134.1 (d, *J* = 7.5 Hz), 121.6 (d, *J* = 3.0 Hz), 97.9 (d, *J* = 7.5 Hz), 62.6 (d, *J* = 96 Hz), 60.0 (d, *J* = 6.0 Hz), 58.9 (d, *J* = 16.5 Hz), 55.2 (d, *J* = 7.5 Hz), 46.6 (d, *J* = 3.0 Hz), 46.5, 39.1 (d, *J* = 49.5 Hz), 38.7, 29.3, 26.0, 22.8 (d, *J* = 55.5 Hz) ,21.2 (d, *J* = 9.0 Hz), 14.6 (d, *J* = 7.5 Hz). MS (ESI/APCI) m/z 380.9 $[M+H]^+$. $[\alpha]_D^{25}$= +45 (c = 0.2, $CHCl_3$). Purity: 95.2%

6.1.21.

*(S)-N-Methyl-N-((2-methyl-6-((3S,5R)-3,4,5-trimethylpiperazin-1-yl)pyrimidin-4-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**31**)*

To a solution of **53** (30 mg, 0.1 mmol) in EtOH (2 mL) was added Et_3N (101 mg, 1.0 mmol) and *cis*-2,6-dimethylpiperazine (57 mg, 0.5 mmol). The reaction mixture was stirred at 80°C overnight, and then quenched with saturated $NaHCO_3$ aqueous solution (20 mL). The aqueous layer was extracted with dichloromethane (20 mL). The organic layer was dried over Na_2SO_4 and concentrated. The resulting residue was diluted with DCE (4 mL) and HCHO/H_2O (37 percent, 0.5 mL) was added, followed by $NaBH(OAc)_3$ (34 mg, 0.16 mmol). The mixture was stirred at room temperature overnight and then quenched with saturated $NaHCO_3$ aqueous solution (50 mL). The aqueous layer was extracted with dichloromethane (30 mL x 3). The combined organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 100/1) to give the desired product (30 mg, 76%) as a colorless oil**.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (s, 1H), 7.36 (d, *J* = 7.2 Hz, 1H), 7.06 (s, 2H), 4.32 (s, 2H), 4.05 (t, *J* = 7.6 Hz, 1H), 3.63 (s, 2H), 2.85-2.76 (m, 1H), 2.75-2.62 (m, 3H), 2.47 (s, 3H), 2.39 (s, 3H), 2.29 (s, 3H), 2.24-2.16 (m, 2H), 2.13-2.07 (m, 1H), 2.05-1.96 (m, 1H), 1.95-1.87 (m, 1H), 1.71-1.65 (m, 1H), 1.18 (t, *J* = 6.0 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.3, 166.6, 162.4, 157.5, 147.5, 136.8, 134.4, 121.8, 97.8, 62.3, 59.0, 57.8 (*J* = 4.5 Hz), 50.4, 39.1, 37.8, 29.5, 26.1, 22.5, 21.5, 18.0 (*J* = 4.5 Hz). MS (ESI/APCI) m/z 394.9 $[M+H]^+$. $[\alpha]_D^{25}$= -20 (c = 0.2, $CHCl_3$). Purity: 99.5%

*6.1.22. Ethyl 2,4-difluoro-3-oxobutanoate (**55**)*

Compound **55** was synthesized with slight modification of the procedure reported in the literature. [33] To a suspension of NaH (60% dispersion in mineral oil, 936 mg, 23 mmol) in 50 mL of ether was added ethyl 2-fluoroacetate (5.0 g, 47 mmol) at room temperature. The mixture was stirred at 40°C for 4 h. The reaction mixture was poured into H_2SO_4 (2 M, 15 mL) and extracted with ether (50 mL x 3).

The organic layers were dried over Na_2SO_4 and concentrated to obtain the desired product (2 g, crude) as a yellow oil, which was used directly in the next step without further purification.

*6.1.23. 5-Fluoro-6-(fluoromethyl)-2-methylpyrimidin-4-ol (**56**)*

A mixture of **55** (1.9 g, 11 mmol), acetamidine hydrochloride (2.2 g, 22 mmol) and EtONa (2.3 g, 34 mmol) in 40 mL of EtOH was stirred at 80°C overnight. 6 N HCl (2 mL) was added and the reaction mixture was concentrated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 3/1 to 1/1) to give the desired product (800 mg, 43%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.07 (br s, 1H), 5.35 (d, *J* = 46.8 Hz, 2H), 2.53 (s, 3H).

*6.1.24. 5-Fluoro-4-(fluoromethyl)-2-methyl-6-(4-methylpiperazin-1-yl)pyrimidine (**57**)*

To a solution of **56** (800 mg, 5.0 mmol), Et_3N (1.5 g, 15 mmol) and *N*-methyl piperazine (750 mg, 7.5 mmol) in 40 mL of MeCN was added PyBOP (2.9 g, 5.5 mmol). The mixture was stirred at reflux overnight. The resulting solution was evaporated to remove MeCN. The residue was diluted with dichloromethane (100 mL) and washed with saturated NaCl aqueous solution (50 mL x 3). The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate) to give the product (1 g, 83%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.36 (d, *J* = 47.2 Hz, 2H), 3.87-3.73 (m, 4H), 2.57-2.50 (m, 4H), 2.49 (s, 3H), 2.33 (s, 3H). MS (ESI/APCI) m/z 242.9 $[M+H]^+$.

*6.1.25. 1-(5-Fluoro-2-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)-N-methylmethanamine (**58**)*

A mixture of **57** (1.5 g, 6.2 mmol) and 2 N methylamine/methanol (6 mL) in *i*-PrOH (15 mL) and H_2O (15 mL) was stirred at 80°C overnight in a sealed tube. The reaction mixture was concentrated, diluted with H_2O (20 mL) and extracted with dichloromethane (30 mL x 3). The combined organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 40/1 to 20/1) to give the product (800 mg, 51%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.80-3.74 (m, 4H), 3.73 (s, 2H), 2.48-2.46 (m, 4H), 2.44 (s, 6H), 2.31 (s, 3H). MS (ESI/APCI) m/z 254.0 $[M+H]^+$.

6.1.26.

*N-((5-Fluoro-2-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine(**21**)*

To a solution of **58** (100 mg, 0.4 mmol), **49** (81 mg, 0.48 mmol) and DIPEA (103 mg, 0.8 mmol) in 5 mL of *i*-PrOH was stirred at 90°C for 3 days. The resulting solution was evaporated to remove MeCN. The residue was diluted with dichloromethane (100 mL) and washed with saturated NaCl aqueous solution (50 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 20/1) to give the product (73 mg, 48%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J* = 4.0 Hz, 1H), 7.34 (d, *J* = 7.6 Hz, 1H), 7.06-7.02 (m, 1H), 4.11-4.00 (m, 1H), 3.81-3.60 (m, 6H), 2.89-2.75 (m, 1H), 2.75-2.62 (m, 1H), 2.50-2.45 (m, 4H), 2.46-2.42 (m, 3H), 2.40 (s, 3H), 2.32 (s, 3H), 2.16-1.96 (m, 3H), 1.76-1.68 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.4 (d, *J* = 9.0 Hz), 157.4, 152.2 (d, *J* = 13.5 Hz), 147.2, 144.5 (d, *J* = 256.5 Hz), 136.6, 134.2, 121.7, 64.5, 55.2, 53.5, 46.3, 46.1, 46.1, 39.2, 29.3, 25.5, 24.7, 21.3. HRMS (ESI): calcd for $C_{21}H_{30}FN_6$ $[M+H]^+$ 385.2516, found 385.2514. Purity: 98.6%

*6.1.27. 4-Hydroxy-2,6-dimethylpyrimidine-5-carbonitrile (**60**)*

A mixture of **59** (5.0 g, 27 mmol), acetamidine hydrochloride (3.9 g, 41 mmol) and K_2CO_3 (11.3 g, 82 mmol) in 80 mL of EtOH was stirred at room temperature overnight. The reaction mixture was acidified pH to 5 by 3 N HCl and then extracted with butyl alcohol (50 mL x 6). The combined organic layer was dried over Na_2SO_4 and concentrated to give the desired product (3.5 g, 87%) as a slight

yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.30 (s, 1H), 2.39 (s, 3H), 2.35 (s, 3H). MS (ESI/APCI) m/z 150.0 $[M+H]^+$.

*6.1.28. tert-Butyl 4-(5-cyano-2,6-dimethylpyrimidin-4-yl)piperazine-1-carboxylate (**61**)*

A mixture of **60** (2.5 g, 16 mmol), 1-Boc-piperazine (4.7 g, 25 mmol), PyBOP (9.6 g, 18 mmol) and Et_3N (5.1 g, 50 mmol) in 60 mL of MeCN was stirred at 80°C overnight. The reaction solution was evaporated to remove most of MeCN. The resulting residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 5/1 to 3/1) to give the desired product (4.3 g, 81%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.94 (s, 4H), 3.55 (s, 4H), 2.57 (s, 3H), 2.50 (s, 3H), 1.47 (s, 9H). MS (ESI/APCI) m/z 317.9 $[M+H]^+$.

*6.1.29. tert-Butyl 4-(6-(chloromethyl)-5-cyano-2-methylpyrimidin-4-yl)piperazine-1-carboxylate (**62**)*

To a solution of **61** (4.0 g, 12 mmol) in 100 mL of dichloromethane was added trichloroisocyanuric acid (2.9 g, 12 mmol) in portions at 0°C. The mixture was stirred at room temperature for 6 h and then quenched with saturated $Na_2S_2O_3$ aqueous solution (20 mL). The aqueous layer was extracted with dichloromethane (50 mL x 3). The combined organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 10/1 to 5/1) to give the desired product (2.4 g, 54%) as a slight yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.58 (s, 2H), 3.99 (s, 4H), 3.57 (s, 4H), 2.56 (s, 3H), 1.48 (s, 9H).

*6.1.30. tert-Butyl 4-(5-Cyano-2-methyl-6-((methyl(5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)pyrimidin-4-yl)piperazine-1-carboxylate (**63**)*

A mixture of *N*-methyl-5,6,7,8-tetrahydroquinolin-8-amine (421 mg, 2.6 mmol), **62** (1.0 g, 2.8 mmol), KI (45 mg, 0.3 mmol) and DIPEA (671 mg, 5.2 mmol) in 10 mL of MeCN was stirred at room temperature overnight. The reaction solution was evaporated to remove most of MeCN. The resulting residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 1/1 to 0/1) to give the desired product (800 mg, 64%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, *J* = 3.6 Hz, 1H), 7.35 (d, *J* = 7.2 Hz, 1H), 7.04 (dd, *J* = 7.2, 4.8 Hz, 1H), 4.15-4.08 (m, 1H), 4.08-3.97 (m, 2H), 3.97-3.88 (m, 4H), 3.02-2.93 (m, 4H), 2.86-2.76 (m, 1H), 2.73-2.63 (m, 1H), 2.53 (s, 3H), 2.28 (s, 3H), 2.22-2.12 (m, 1H), 2.10-1.92 (m, 2H), 1.76 (s, 10H).

*6.1.31. 2-Methyl-4-((methyl(5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-6-(4-methylpiperazin-1-yl)pyrimidine-5-carbonitrile (**22**)*

To a solution of **63** (600 mg, 1.3 mmol) in 2 mL of dichloromethane was added CF_3COOH (1 mL). The mixture was stirred at room temperature for 2 h and then evaporated to give the product (700 mg, crude) as a red oil. To a solution of the crude product in 1 mL of methanol was added $HCHO/H_2O$ (37 percent, 1 mL) and $NaBH_3CN$ (125 mg, 2.0 mmol). The mixture was stirred at room temperature for 2 h and then quenched with saturated $NaHCO_3$ aqueous solution (10 mL). The aqueous layer was extracted with dichloromethane (10 mL x 3). The combined organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by Al_2O_3 column chromatography (petroleum ether/ethyl acetate = 5/1 to 0/1) to give the desired product (200 mg, 39%) as a slight yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, *J* = 4.0 Hz, 1H), 7.35 (d, *J* = 7.2 Hz, 1H), 7.04 (dd, *J* = 8.0, 4.8 Hz, 1H), 4.15-4.08 (m, 1H), 4.08-3.93 (m, 6H), 2.87-2.75 (m, 1H), 2.75-2.62 (m, 1H), 2.55-2.45 (m, 7H), 2.33 (s, 3H), 2.23 (s, 3H), 2.08-1.90 (m, 3H), 1.77-1.64 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 168.5, 162.4, 157.5, 147.0, 136.7, 134.3, 121.7, 117.4, 87.6, 64.8, 61.0, 55.0, 46.5, 46.1, 38.1, 29.3, 26.6, 26.4, 21.4. HRMS (ESI): calcd for $C_{22}H_{30}N_7$ $[M+H]^+$ 392.2563, found 392.2559. Purity: 98.2%

6.2. *In vitro* biological assays

6.2.1. HPB-ALL CXCR4 competitive binding assay [13]

HPB-ALL cells were maintained in RPMI-1640 (Gibico) supplemented with 10% FBS (Hyclone). APC-conjugated anti-human CXCR4 was from Sungene. EC_{80} (0.83 μg/mL) was first determined for 12G5 binding to CXCR4. Then the compounds for testing were added into 96-well plates serially diluted at a ratio of 1:3. Cells were washed once with ice-cold assay buffer (DPBS+2% HI-FBS) and then re-suspended in the same buffer at a final concentration of 1×10^6/mL. Cell suspension was then added into the wells and with the addition of APC-conjugated anti-human CXCR4 clone 12G5 at its EC_{80} determined. The mix of cell, compounds and APC-conjugated anti-human CXCR4 were incubated at 4 ºC for 3 h before addition of 100 μL of 4% PFA. Cells were then washed once and resuspended in assay buffer and examined by FACS.

6.2.2. FLIPR Tetra calcium mobilization assay [13]

The FLIPR Tetra calcium mobilization assay was performed by HD Bioscience. Briefly, The Molecular Devices, Fluorescent Imaging Plate Reader (FLIPR) Tetra was used in this assay. Excitation was achieved through unique placement of LED's within the instrument and emission captured by a CCD camera (EMCCD camera for FI and ICCD camera for luminescence). The homogeneous FLIPR Calcium 4 assay kit from Molecular Devices was used as the fluorescence reagent. Compounds were solubilized in 100% dimethyl sulfoxide (DMSO) to a concentration of 30 mM. A 10-point, 4-fold, intermediate dilution series was created in 100% DMSO with a top concentration of 400 μM and a bottom concentration of 0.001 μM. A near assay ready, direct dilution plate (ddNARP) was prepared from this compound dilution plate by transferring 1 μL of each dilution of compound in 100% DMSO to a Greiner#781201 plate. In addition, each ddNARP plate also contained positive and negative control wells to define the upper and lower limits for the assay signal. The final assay concentration range of compound was 1 μM to 0.0035 nM in 0.5% DMSO. Human CD^{4+} T-Cells were isolated from human whole blood and subsequently activated and expanded using a CD3/CD28 expansion kit (Life Technologies). The cells were frozen in ThermoFisher-formulated Recovery Cell Culture Freezing Medium containing 10% Dimethyl sulfoxide (DMSO) and 10% Fetal Bovine Serum (FBS) (ThermoFisher Catalog No. 10100147). When used, cells were resuspended using room temperature 1X HBSS/20 mM HEPES/0.005% P-104 assay buffer, adjusted the volume of the suspension to achieve a cell concentration of 2.5×10^6 cells/mL. 2X Calcium 4 dye (20 μL/well) were added and the mixture were centrifuged briefly (~10 s) and stopped when it reached 1000 rpm. The plates were allowed to equilibrate before compounds were added. After 20 min, CXCL12 (EC_{80}, 5 nM) was added to the plates. The raw data were analyzed using Abase. The percent (%) effect at each concentration of compound was calculated by Abase and was based on and relative to the amount of calcium produced in the positive and negative control wells contained within each assay plate. The concentrations and % effect values for tested compounds were plotted by Abase and the concentration of compound required for 50% effect (IC_{50}) was determined with a four-parameter logistic dose response equation.

6.2.3. CXCR1 functional assay

Recombinant HEK293 Cells with CXCR1 over-expression were dissociated using Trypsin-EDTA at 37°C for 3 mins, before fresh culture medium was added onto cells to stop Trypsin-EDTA. The cells were centrifuged at 1200 rpm for 3 mins, and then resuspended using assay buffer. Cell number was

counted using Countess (Invitrogen). PBS was added to adjust cell density to 2.7 x 10^5/ml.

IL-8 was serial diluted in LDV (low dead volume microplate) with 100 µM top dose, 4-fold dilution, 10 points, and dispensed 100 nL/well into assay plate. Cells (7.5 µL, 2000/well) were added, and the mixture was incubated at room temperature for 15 min. Forskolin (4.4 µM, 2.5 µL) was added to the assay plate, and the mixture was incubated at room temperature for another 15 min. cAMP-d2 (5 µL) and Eu-Anti-cAMP working solutions (5 µL) were added, and the mixture was incubated at room temperature for 60 min. The raw data were read on Envision (665 nm/615 nm).

Compound **23** and Navarixin were serial diluted in LDV with 10 mM top dose, 4-fold dilution, 10 points, and dispensed 100 nL/well into assay plate. Cells (7.5 µL, 2000/well) were added, and the mixture was incubated at room temperature for 15 min. A solution of Forskolin and IL-8 in assay buffer (2.5 µL, 4.4 µM for Forskolin and 100 nM for IL-8) was added to the assay plate, and the mixture was incubated at room temperature for another 15 min. cAMP-d2 (5 µL) and Eu-Anti-cAMP working solutions (5 µL) were added, and the mixture was incubated at room temperature for 60 min. The raw data were read on Envision (665 nm/615 nm).

6.2.4. CCR6 functional assay

The CCR6 functional assay was performed by HD Bioscience. Briefly, The Molecular Devices, Fluorescent Imaging Plate Reader (FLIPR) Tetra was used in this assay. Excitation was achieved through unique placement of LED's within the instrument and emission captured by a CCD camera (EMCCD camera for FI and ICCD camera for luminescence). The homogeneous FLIPR Calcium 4 assay kit from Molecular Devices was used as the fluorescence reagent. Compounds were solubilized in 100% dimethyl sulfoxide (DMSO) to a concentration of 30 mM. A 10-point, 4-fold, intermediate dilution series was created in 100% DMSO with a top concentration of 4 mM and a bottom concentration of 0.01 µM. A near assay ready, direct dilution plate (ddNARP) was prepared from this compound dilution plate by transferring 1 µL of each dilution of compound in 100% DMSO to a Greiner#781201 plate. In addition, each ddNARP plate also contained positive and negative control wells to define the upper and lower limits for the assay signal. The final assay concentration range of compound was 10 µM to 0.035 nM in 0.5% DMSO. Human CD^{4+}, CCR6 enriched T-Cells were previously activated, expanded and subsequently frozen in ThermoFisher-formulated Recovery Cell Culture Freezing Medium containing 10% Dimethyl sulfoxide (DMSO) and 10% Fetal Bovine Serum (FBS) (ThermoFisher Catalog No. 10100147). When used, cells were resuspended using room temperature 1X HBSS/20 mM HEPES/0.005% P-104 assay buffer, adjusted the volume of the suspension to achieve a cell concentration of 2.5 × 10^6 cells/mL. 2X Calcium 4 dye (20 µL/well) were added and the mixture were centrifuged briefly (~10 s) and stopped when it reached 1000 rpm. The plates were allowed to equilibrate before compounds and CCL20 were added to the plates. The raw data were analyzed using Abase. The percent (%) effect at each concentration of compound was calculated by Abase and was based on and relative to the amount of calcium produced in the positive and negative control wells contained within each assay plate. The concentrations and % effect values for tested compounds were plotted by Abase and the concentration of compound required for 50% effect (IC_{50}) was determined with a four-parameter logistic dose response equation.

6.2.5. Matrigel invasion assay

The human breast cancer cell line MDA-MB-231 was purchased from ATCC (Manassas, VA). MDA-MB-231cell line was cultured in DMEM medium supplemented with 10% heat-inactivated fetal

bovine serum, 100 units/ml of penicillin and 100 mg/ml of streptomycin. Cells were cultured at 37 °C in a humidified atmosphere of 20% O_2/5% CO_2. All cultures were monitored routinely and found to be free of contamination by mycoplasma or fungi, discarded after three months, and new lines propagated from frozen stocks.

Matrigel invasion assays were carried out in modified Boyden chambers with filter inserts with 8-μm pores in 24-well plates (Corning, NY, USA). The surfaces of the filters were coated with 50mg/L ice-cold Matrigel (Matrigel basement membrane matrix, BD Bioscience, NJ, USA). The lower chamber was filled with medium containing 10% serum. The target compounds (100 nM) and the human breast cancer cell line MDA-MB-231 cells (4×10^4 cells/well) were added to the upper chamber of a vessel and CXCL12 was added in the lower chamber as a chemoattractant in serum free medium. After 24 h incubation, the filters were gently removed from the chambers, and the cells on the upper surface were removed by wiping with a cotton swab. Cells that had invaded to the lower surface areas were fixed with ice cold methanol, stained with crystal violet, and counted in 10 randomly selected fields under a microscope (100×). Results shown are representative of three independent experiments.

6.2.6. Cell viability assay

The cell viability was analyzed by using the CellTiter-Glo Luminescent Cell Viability Assay kit following the manufacturer's instructions (Promega). Luminescence was measured with SpectraMax i3x (Molecular Devices).

6.3. Preliminary in vitro safety and DMPK test

6.3.1. Evaluation of CYP inhibitory potency [34]

Inhibitory potency of test compounds against human P450 enzymes was investigated in human liver microsomes. The five major CYP isoforms and their respective probe substrates were as follows: CYP3A4 (midazolam, 1μM), CYP2D6 (dextromethorphan, 5 μM), CYP1A2 (phenacitine, 20 μM), CYP2C9 (tolbutamide, 100 μM), and CYP2C19 (S-mephenytoin, 40 μM). All probe substrates were used at concentrations near or below their K_ms (Michaelis constant). Triplicate incubations were conducted in the 200 μL incubation mixtures at 37 °C. The mixture contained HLM (0.2 mg/mL), phosphate buffer (100 mM, pH 7.4), NADPH (1 mM), tested compounds (10 μM), and individual CYP probe substrate. The mixtures were pre-incubated for 10 min to allow inhibitor/enzyme interactions before the initiation of the reaction with NADPH. After 10 min, reactions were terminated by the addition of 100 μL of cold acetonitrile containing an appropriate internal standard. Samples were then centrifuged and injected into the LCMS/MS apparatus to quantify the concentrations of specific metabolites formed by individual CYP enzymes.

6.3.2. hERG assays [35,36]

Compound **23** was evaluated for block of the hERG potassium channel using CHO cells stably expressing the hERG gene and the QPatch platform (Sophion, Ballerup, Denmark). K tail currents were measured at −50 mV following a 500 ms depolarization to +20 mV from a holding voltage of −80 mV. The external solution contained 4 mM K^+, 1 mM Mg^{2+}, and 2 mM Ca^{2+}. Compound effects were quantified 4 min after application to the cells. Pulses were elicited every 20 s.

6.3.3. Metabolic stability test [34]

To test the metabolic stability of compound **23** in vitro, each compound was incubated with rat liver microsomes in the presence of NADPH. The reaction mixtures include NADPH (1 mM), microsomes (0.2 mg/mL), phosphate buffer (100 mM, pH 7.4), and test compounds (1 μM). The reaction was initiated by the addition of NADPH after a 10 min pre-incubation. The incubations were taken at three time points (0, 30, 60 min) and placed into 100 μL of cold acetonitrile with internal standard. The samples were then centrifuged at 13,000 rpm for 10 min and the supernatants were subjected to LC-MS/MS analysis. The in vitro kinetic parameters were calculated using the following equations:

$$t_{1/2} = \frac{0.693}{k} \quad (1)$$

Where k is the slope from linear regression analysis of the natural Ln percent remaining of test compound as a function of incubation time.

$$Cl_{int,\,in\,vitro} = \frac{k}{\text{mg of protein per ml incubation}} \times \frac{\text{mg of protein}^{a}}{\text{g of liver}} \times \frac{\text{g of liver}}{\text{kg of body weight}}^{b} \quad (2)$$

Where a is 20 mg/g for mouse and human, b is 87.5 g/kg for mouse and 25.7 g/kg for human.

$$Cl_{hepatic} = \frac{Q_h \times Cl_{int,\,in\,vitro}}{Q_h + Cl_{int,\,in\,vitro}} \quad (3)$$

Where Q_h is the hepatic blood flow (90 mL/min for mouse and 20.7 mL/min for human).

6.3.4. Caco-2 permeability study [35,37]

The Caco-2 permeability study was performed by ChemPartner. Caco-2 cells were obtained from American Tissue Culture Collection (Rockville, MD). The cells were maintained in Modified Eagle's medium (MEM), containing 10% heat-inactivated fetal bovine serum, and 1% non-essential amino acids, in CO_2 at 37°C. Cells were seeded on polycarbonate filter inserts (Millipore, CAT#PSHT 010 R5).

The cells were cultured for 21–28 days prior to the transport experiments. The transepithelial electric resistance and Lucifer Yellow permeability were checked before and after the assay. Compound**s** were dissolved at 10 mM in 100% dimethyl sulfoxide (DMSO) and diluted for studies in Hanks Balanced Salt Solution (HBSS, Invitrogen, Cat# 14025-092) with 25 mM HEPES, pH 7.4. Compounds were tested at 10 μM, and in both the apical-to-basolateral (A-B) and basolateral-to-apical (B-A) directions, and were conducted at 37°C for 90 min. At the end of incubation, donor samples were diluted 10-fold by assay buffer, then 60 μL of receiver and diluted-donor samples were mixed with 60 μL of acetonitrile, and analyzed by LC-MS/MS. The concentrations of the compounds were quantified by standard curve.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at

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Figure Captions

Fig. 1 Examples of small molecule CXCR4 antagonists reported in the literature

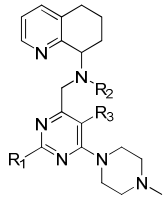
Fig. 2 Molecular docking and structural optimization proposal. (a) The predicted conformation of compound A in the binding site of CXCR4, the residues around 5 Å of compound A are colored in green and the key residues are labeled in pink. (b) The interaction pattern between compound A and CXCR4. (c) Proposed SAR study plan.

Fig. 3 (a) The nearby residues within 5 Å of the compound **A** in the binding pocket of CXCR4 receptor; schematic representation of the interaction patterns between (b) compound **4**, (c) compound **12** and CXCR4 receptor (PDB ID: 3ODU).

Fig. 4 Compound **23** is a potent CXCR4 antagonist, but is inactive to CXCR1 and CCR6. (a) CXCR4 dose response curve for SDF-1 (CXCL12) stimulation and inhibition curves of compound **23**/AMD3100 (positive control for CXCR4) based on a calcium flux assay. (b) CXCR1 dose response curve for IL-8 stimulation and inhibition curves of compound **23**/Navarixin (positive control for CXCR1) based on a functional cAMP assay. (c) CCR6 dose response curve for CCL20 stimulation and inhibition curves of compound **23**/PF-9654-00 (positive control for CCR6) based on a calcium flux assay.

Fig. 5 Effect of compound **23** on matrigel invasion of MDA-MB-231 cells and cytotoxicity assessment of **23** in the cultured human and murine cells. (a) Photo images of matrigel 22 h after invasion experiment. (b) Quantification of transwell analysis of cell invasion experiment. Data represent mean value ± standard deviation. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Magnification: 4x. (c) Effect of compound **23** on proliferation of human and murine cells.

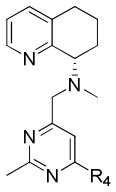
Table 1. SAR for R_1, R_2, and R_3

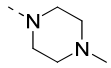
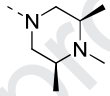
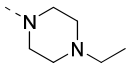
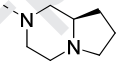
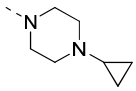
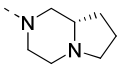
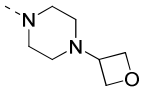
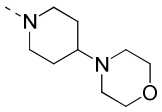
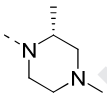
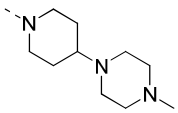
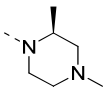
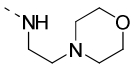
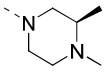
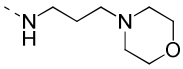
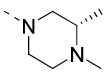
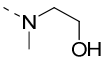


Cmpd	R_1	R_2	R_3	12G5 Binding IC_{50} (nM)[a]	clogP[b]	pKa[c]	Cmpd	R_1	R_2	R_3	12G5 Binding IC_{50} (nM)[a]	clogP[b]	pKa[c]
1	MeO	Me	H	25 ± 2	2.35	6.5	**12**	Me	Me	H	12 ± 3	2.06	7.2
2	EtO	Me	H	78 ± 14	2.72	6.5	**13**	Et	Me	H	25 ± 4	2.63	7.3
3	*i*-PrO	Me	H	151 ± 74	3.08	6.7	**14**	*i*-Pr	Me	H	256 ± 89	3.11	7.1
4	NH_2	Me	H	23 ± 6	1.77	6.7	**15**	*c*-Pr	Me	H	23 ± 4	2.42	7.4
5	MeNH	Me	H	58 ± 15	2.57	8.7	**16**	Ph	Me	H	2891 ± 202	4.08	6.3
6	EtNH	Me	H	105 ± 38	2.94	8.8	**17**		Me	H	1294 ± 505	2.46	6.3
7	*i*-PrNH	Me	H	930 ± 375	3.24	8.9	**18**[d]	CF_3	Me	H	293 ± 52	2.97	5.7
8	$(Me)_2N$	Me	H	> 10000	2.93	9.0	**19**	Me	Et	H	17 ± 4	2.43	8.2
9		Me	H	792 ± 186	2.30	8.9	**20**	Me		H	51 ± 11	2.93	8.3
10		Me	H	2442 ± 190	2.77	9.9	**21**	Me	Me	F	12 ± 4	2.15	6.1
11		Me	H	> 10000	3.33	9.1	**22**	Me	Me	CN	33 ± 5	1.74	5.7
A				88 ± 16	1.97	6.7	**AMD3100**				303 ± 24		

[a] Inhibition of luminescence signaling in HPB-ALL CXCR4 competitive binding assay. Data are expressed as geometric mean values of at least two runs ± the standard error measurement (SEM). [b] Calculated by Molinspiration. [c] Calculated by ACD/Labs 6.0. [d] Compound **18** was synthesized as (*S*)-stereoisomer.

Table 2. SAR forR_4



Cmpd	R_4	12G5 Binding IC_{50} (nM)[a]	clogP[b]	pKa[c]	Cmpd	R_4	12G5 Binding IC_{50} (nM)[a]	clogP[b]	pKa[c]
23		8.8 ± 1.0	2.06	7.2	**31**		8.3 ± 3.9	2.72	7.2
24		10 ± 1	2.43	7.2	**32**		30 ± 17	2.45	8.0
25		70 ± 12	2.42	7.2	**33**		18 ± 9	2.45	8.0
26		130 ± 7	1.89	7.2	**34**		101 ± 45	2.43	7.3
27		21 ± 7	2.39	7.2	**35**		96 ± 12	2.48	7.7
28		14 ± 7	2.39	7.2	**36**		66 ± 24	1.80	7.0
29		8.3 ± 4.7	2.39	7.2	**37**		36 ± 18	2.07	7.5
30		14 ± 7	2.39	7.2	**38**		> 10000	1.53	6.2
A		88 ± 16	1.97	6.7	**AMD3100**		303 ± 24		

[a] Inhibition of luminescence signaling in HPB-ALL CXCR4 competitive binding assay. Data are expressed as geometric mean values of at least two runs ± the standard error measurement (SEM). [b] Calculated by Molinspiration. [c] Calculated by ACD/Labs 6.0.

Table 3. Preliminary *in vitro* safety and DMPK evaluation of compound 23

Cmpd	CYP Inhibition (%)[a]		hERG IC_{50}	Caco-2		PPB (%)			RLM[b]	
	3A4	2D6	(μM)	Papp(A−B) (10^{-6} cm/s)	efflux ratio (B−A)/(A−B)	mouse	rat	human	Cl_{int} (mL/min/kg)	$t_{1/2}$ (min)
23	48	57	8	27	0.86	89	82	99	1567	1.6
A[c]	9	3	> 30	15	1.5	ND[d]	ND	ND	2256	1.1

[a] All compounds were tested at 10 μM concentration. [b] RLM = Rat liver microsomes. All compounds were tested at 1 μM concentration. [c] Data of compound A has been reported previously [13]. [d] ND = not determined.

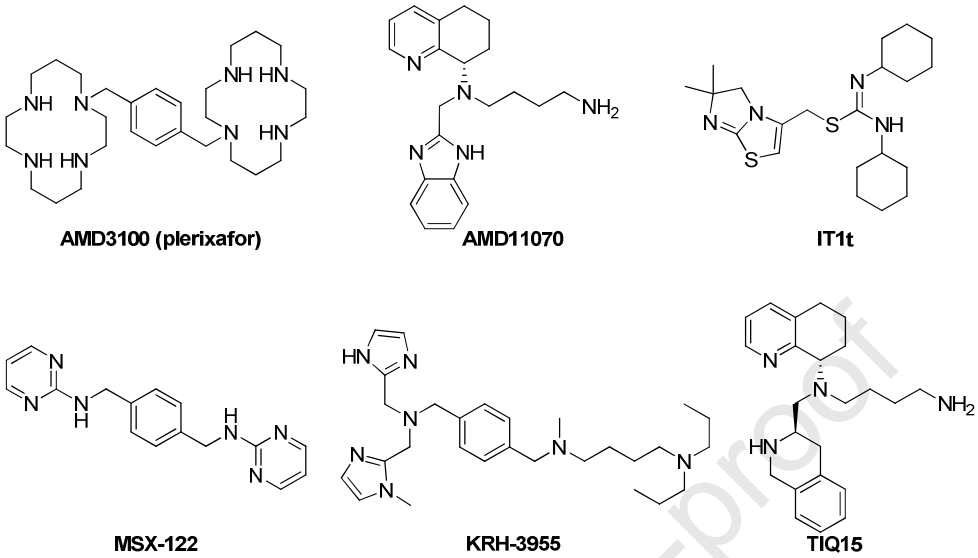


Figure 1

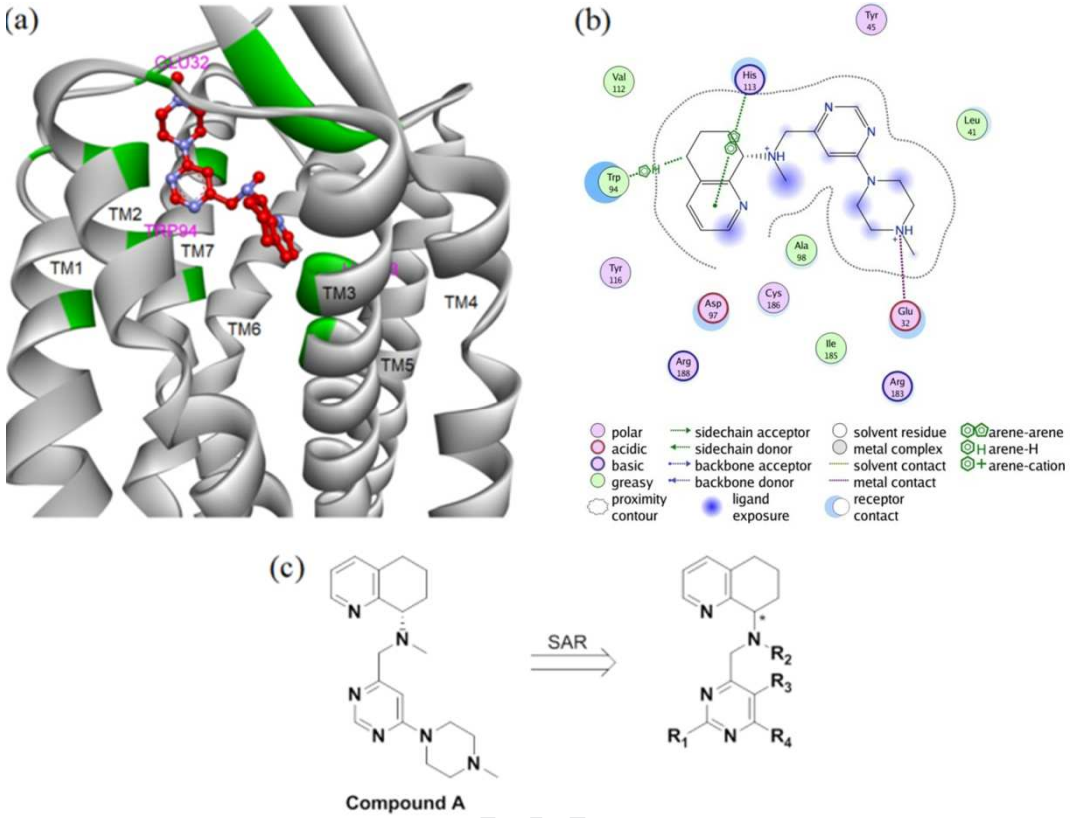


Figure 2

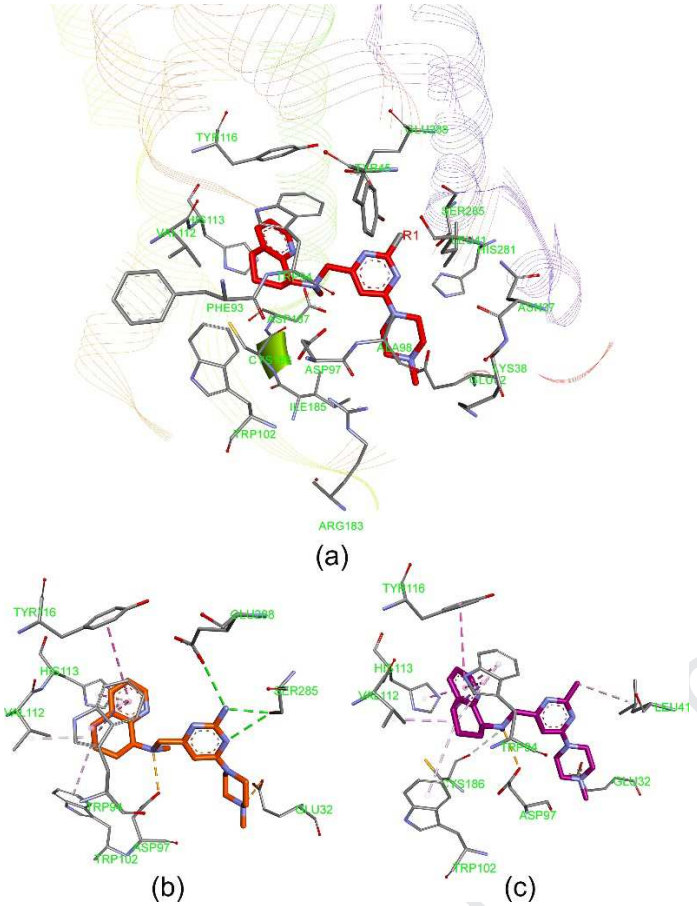


Figure 3

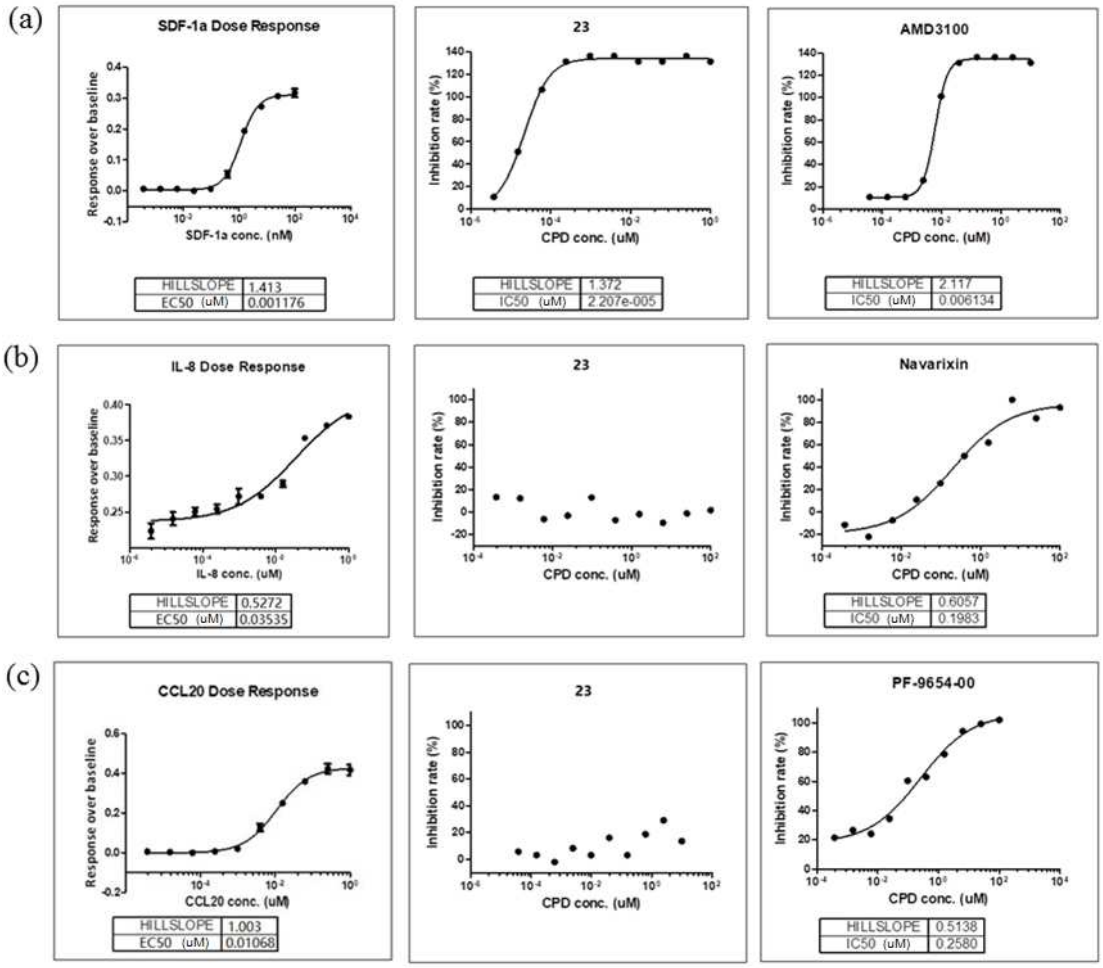

(a)
SDF-1a Dose Response
Response over baseline
SDF-1a conc. (nM)
HILLSLOPE 1.413
EC50 (uM) 0.001176
23
Inhibition rate (%)
CPD conc. (uM)
HILLSLOPE 1.372
IC50 (uM) 2.207e-005
AMD3100
Inhibition rate (%)
CPD conc. (uM)
HILLSLOPE 2.117
IC50 (uM) 0.006134
(b)
IL-8 Dose Response
Response over baseline
IL-8 conc. (uM)
HILLSLOPE 0.5272
EC50 (uM) 0.03535
23
Inhibition rate (%)
CPD conc. (uM)
Navarixin
Inhibition rate (%)
CPD conc. (uM)
HILLSLOPE 0.6057
IC50 (uM) 0.1983
(c)
CCL20 Dose Response
Response over baseline
CCL20 conc. (uM)
HILLSLOPE 1.003
EC50 (uM) 0.01068
23
Inhibition rate (%)
CPD conc. (uM)
PF-9654-00
Inhibition rate (%)
CPD conc. (uM)
HILLSLOPE 0.5138
IC50 (uM) 0.2580

Figure 4

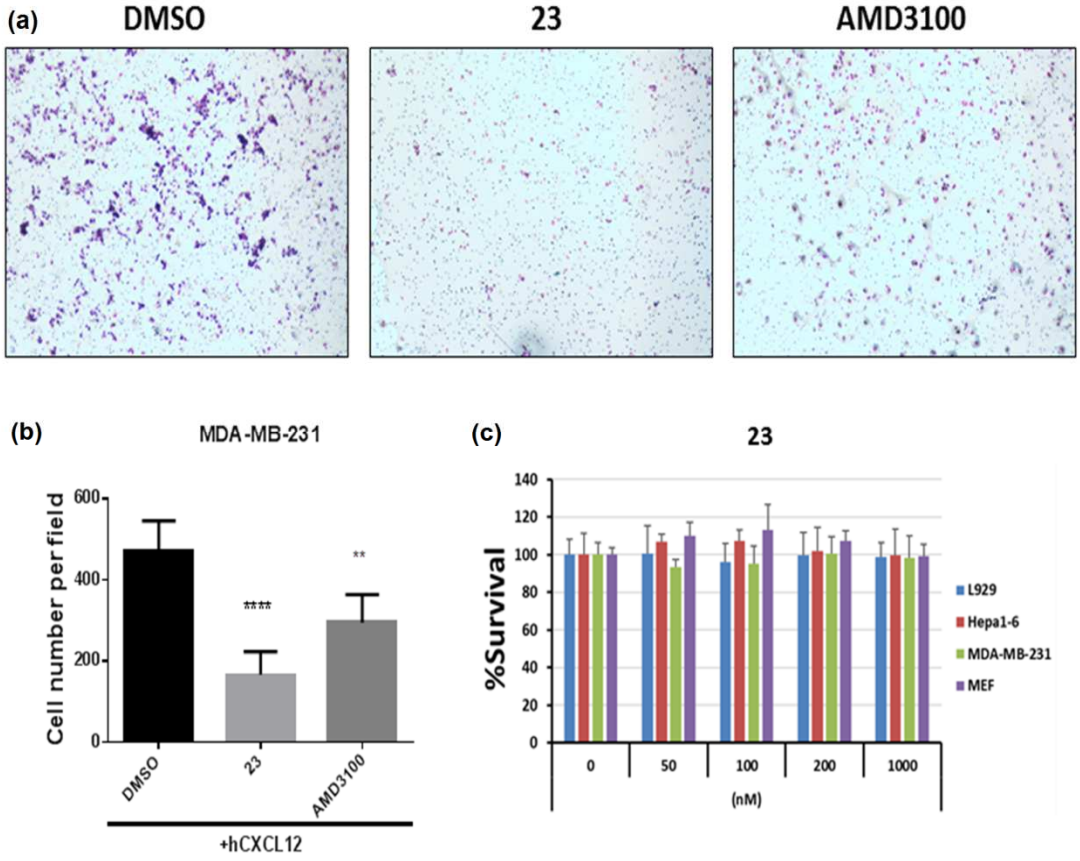
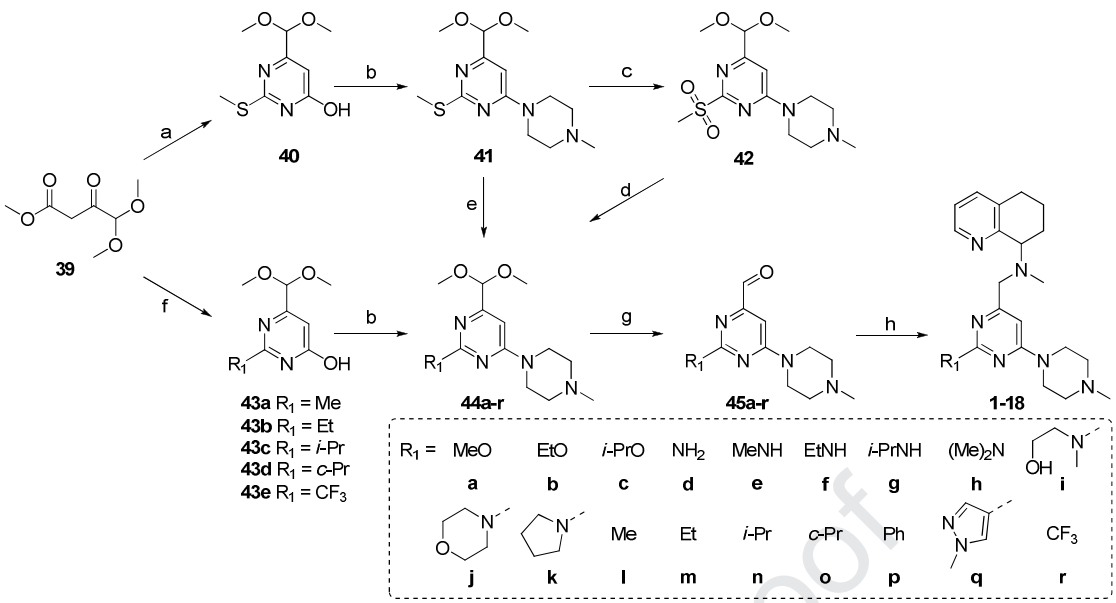
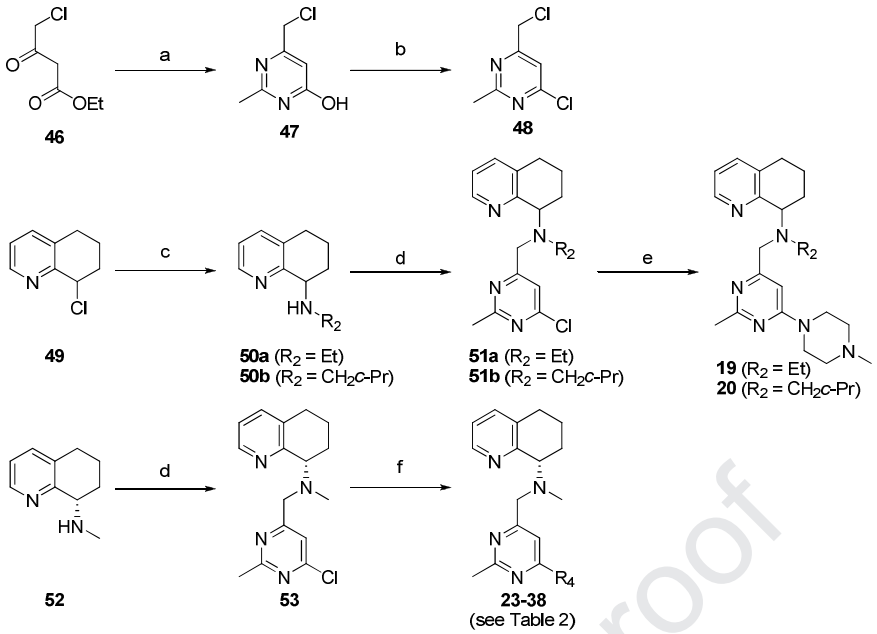


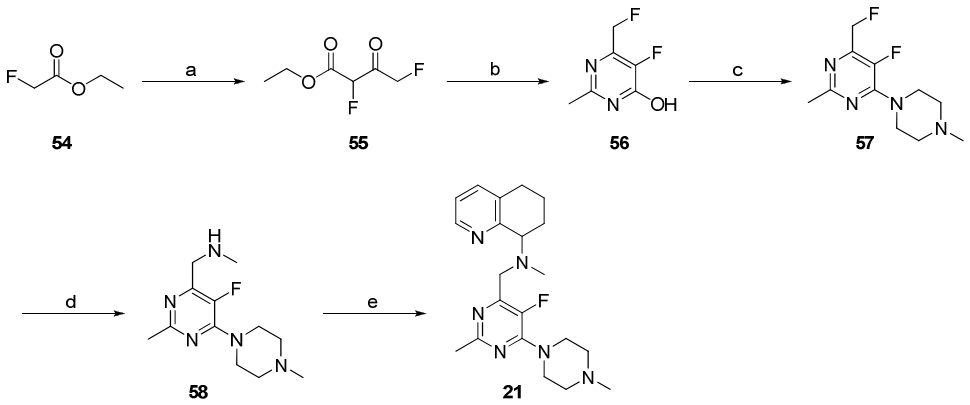
Figure 5



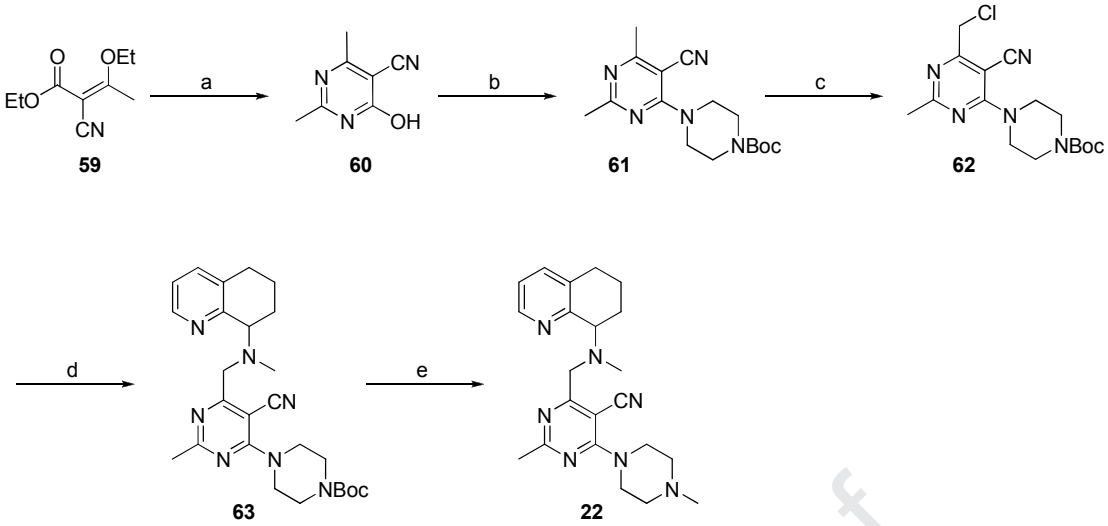
Scheme 1. Reagents and conditions: (a) 2-methyl-2-thiopseudourea sulfate, K_2CO_3, H_2O, rt, overnight; (b) *N*-methyl piperazine, PyBOP, Et_3N, MeCN, reflux, overnight; (c) Oxone, THF, H_2O, rt, 4 h; (d) for **44a** and **44b**: MeONa/MeOH or EtONa/EtOH, reflux, overnight; for 44c: NaH, isopropanol, reflux, overnight; for **44d-k**: corresponding amine, THF, reflux, overnight; (e) for **44p** and **44q**: corresponding boronic acid, $Pd(PPh_3)_4$, CuTC, THF, 80 °C, 12 h; (f) corresponding amidine, K_2CO_3, H_2O, rt, overnight; (g) 20% H_2SO_4, reflux, overnight; (h) for **1-17**: (±)-*N*-methyl-5,6,7,8-tetrahydroquinolin-8-amine, $NaBH(OAc)_3$, AcOH, DCE, rt, overnight; for **18**: (*S*)-*N*-methyl-5,6,7,8-tetrahydroquinolin-8-amine, $NaBH(OAc)_3$, AcOH, DCE, rt, overnight.



Scheme 2. Reagents and conditions: (a) acetamidine hydrochloride, DBU, EtOH, rt, overnight; (b) $POCl_3$, reflux, 30 min; (c) corresponding amine, THF, 80 °C, overnight; (d) **48**, DIPEA, KI, MeCN, rt, overnight; (e) *N*-methyl piperazine, Et_3N, EtOH, 80 °C, overnight; (f) for **23-26**, **29**, **30**, and **32-38**: corresponding amine, Et_3N, EtOH, 80 °C, overnight; for **27** and **28**: i) *tert*-butyl (*R*)-3-methylpiperazine-1-carboxylate or *tert*-butyl (*S*)-3-methylpiperazine-1-carboxylate, Et_3N, EtOH, 80 °C, overnight; ii) HCl/ethyl acetate, rt, overnight; iii) HCHO, $NaBH(OAc)_3$, DCE, rt, overnight; for **31**: i) *cis*-2,6-dimethylpiperazine, Et_3N, EtOH, 80 °C, overnight; ii) HCHO, $NaBH(OAc)_3$, DCE, rt, overnight.



Scheme 3. Reagents and conditions: (a) NaH, Et_2O, reflux, 4 h; (b) acetamidine hydrochloride, EtONa, EtOH, 80 °C, overnight; (c) *N*-methyl piperazine, PyBOP, Et_3N, MeCN, reflux, overnight; (d) 2 N CH_3NH_2/MeOH, *i*-PrOH, H_2O, 80 °C, overnight; (e) **49**, DIPEA, *i*-PrOH, 90 °C, 3 d.



Scheme 4. Reagents and conditions: (a) acetamidine hydrochloride, K_2CO_3, EtOH, rt, overnight; (b) *N*-Boc-piperazine, PyBOP, Et_3N, MeCN, 80 °C, overnight; (c) trichloroisocyanuric acid, CH_2Cl_2, rt, 6 h; (d) (±)-*N*-methyl-5,6,7,8-tetrahydroquinolin-8-amine, KI, DIPEA, MeCN, rt, overnight; (e) (i) TFA, CH_2Cl_2, rt, 2 h; (ii) HCHO/H_2O, $NaBH_3CN$, MeOH, rt, 2 h.

Highlights

(1) Structural optimization of aminopyrimidine-based CXCR4 antagonists were reported.
(2) Compound **23** competes with APC-conjugate 12G5 for CXCR4 binding (IC_{50} = 8.8 nM).
(3) Compound **23** inhibits CXCL12 induced cytosolic calcium increase (IC_{50} = 0.02 nM).
(4) Compound **23** inhibits CXCR4/CXLC12 mediated chemotaxis in a matrigel assay.
(5) Compound **23** demonstrates good physicochemical properties and in vitro safety profiles.

The authors of the manuscript entitled “Structural Optimization of Aminopyrimidine-Based CXCR4 Antagonists” (Ms. Ref. No.: EJMECH-D-19-02294) declare no conflicts of interest to this submitted work.