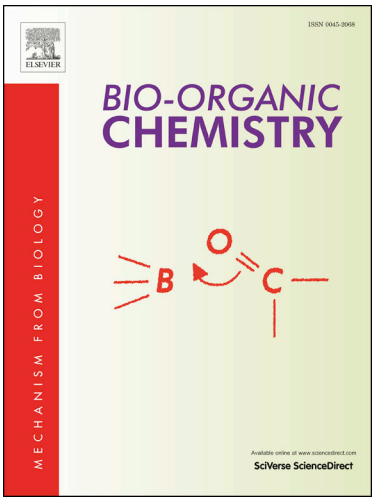
Journal Pre-proofs



Design, Synthesis, and Evaluation of Novel CXCR4 Antagonists Based on an Aminoquinoline Template

Yu Lin, Zhanhui Li, Chen Xu, Kaijiang Xia, Shuwei Wu, Yongjin Hao, Qing Yang, Haikuo Ma, Jiyue Zheng, Lusong Luo, Fang Zhu, Sudan He, Xiaohu Zhang

PII: S0045-2068(20)30139-5
DOI: https://doi.org/10.1016/j.bioorg.2020.103824
Reference: YBIOO 103824

To appear in: *Bioorganic Chemistry*

Received Date: 19 January 2020
Revised Date: 23 February 2020
Accepted Date: 5 April 2020

Please cite this article as: Y. Lin, Z. Li, C. Xu, K. Xia, S. Wu, Y. Hao, Q. Yang, H. Ma, J. Zheng, L. Luo, F. Zhu, S. He, X. Zhang, Design, Synthesis, and Evaluation of Novel CXCR4 Antagonists Based on an Aminoquinoline Template, *Bioorganic Chemistry* (2020), doi: https://doi.org/10.1016/j.bioorg.2020.103824

Design, Synthesis, and Evaluation of Novel CXCR4 Antagonists Based on an Aminoquinoline Template

Yu Lin,[a,1] Zhanhui Li,[a,1] Chen Xu,[a] Kaijiang Xia,[a] Shuwei Wu,[a] Yongjin Hao,[a] Qing Yang,[a] Haikuo Ma,[a] Jiyue Zheng,[a,*] Lusong Luo,[b] Fang Zhu,[c,d] Sudan He,[c,d,*] and Xiaohu Zhang [a,*]

[a] Jiangsu Key Laboratory of Neuropsychiatric Diseases and College of Pharmaceutical Sciences, Soochow University, Suzhou, Jiangsu 215123, P. R. China

[b] BeiGene (Beijing) Co., Ltd., No. 30 Science Park Road, Zhongguancun Life Science Park, Beijing 102206, P. R. China

[c] Cyrus Tang Hematology Center, Jiangsu Institute of Hematology and Collaborative Innovation Center of Hematology, Soochow University, Suzhou 215123, P.R. China.

[d] Center of Systems Medicine, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medial College, Beijing; Suzhou Institute of Systems Medicine, Suzhou, 215123 Jiangsu, P. R. China

[1] These two authors contribute equally to this study.

* Corresponding author. Tel.: +86 512 65880380; fax: +86 512 65880380; email: xiaohuzhang@suda.edu.cn; jyzheng@suda.edu.cn; hesudan2018@163.com

Abstract: The chemokine receptor CXCR4 has been explored as a drug target due to its involvement in pathological conditions such as HIV infection and cancer metastasis. Here we report the structure-activity relationship study of novel CXCR4 antagonists based on an aminoquinoline template. This template is devoid of the chiral center in the classical tetrahydroquinoline (THQ) ring moiety and therefore can be easily synthesized. A number of potent CXCR4 antagonists were identified, exemplified by compound **3**, which demonstrated excellent binding affinity with CXCR4 receptor (IC_{50} = 57 nM) and inhibited CXCL12 induced cytosolic calcium increase (IC_{50} = 0.24 nM). Furthermore, compound **3** potently inhibited CXLC12/CXCR4 mediated cell migration in a transwell invasion assay. The simplified synthetic approach combined with good physicochemical properties (e.g. MW 362, clogP 2.1, PSA 48, pKa 7.0 for compound **3**) demonstrate the potential of this aminoquinoline template as a novel scaffold to develop CXCR4 antagonists.

Keywords: aminoquinoline, antagonist, Chemokine, CXCL12, CXCR4, GPCR

Abbreviations: APC, allophycocyanin; BINAP, 1.1'-Binaphthyl-2.2'-diphemyl phosphine; Boc, tert-butyloxycarbonyl; CCD, charge coupled device; CYP, cytochrome P450; DBU, 1,8-Diazabicyclo[5.4.0]undec-7-ene; DCE, 1,2-dichloroethane; DIPEA, *N*,*N*-diisopropylethylamine; DMF, N,N-dimethyl formamide; 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; HI-FBS, heat inactivated FBS; HIV, human immunodeficiency virus; ICCD, intensified CCD; MEF, mouse embryonic fibroblasts; Oxone, Potassiumperoxomonosulfate; PK, pharmackinetices; PPB, Plasma protein binding; PyBOP, Benzotriazol-1-yl-

oxytripyrrolidinophosphonium hexafluorophosphate; RLM, rat liver microsomes; rt, room temperature; SAR, structure-activity-relationship; SDF-1, stromal cell-derived factor-1; selectfluor, 1-Chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate); SEM, standard error measurement; TBS, *tert*-butyldimethylsilyl; THQ, tetrahydroquinoline; TIQ, tetrahydroisoquinoline; TLC, thin-layer chromatography.

1. Introduction

Chemokines are a family of small proteins with chemotactic functions. Chemokines bind to specific G-protein coupled receptors (chemokine receptors) to mediate their biological activities. One of the most extensively studied chemokine receptors is CXCR4 receptor. CXCR4 was first identified as a co-receptor utilized by the Human Immunodeficiency Virus (HIV) to gain cellular entry [1]. In addition to this important function, CXCR4 and its endogenous ligand CXCL12 (also known as stromal cell-derived factor-1, or SDF-1) regulate a broad range of physiological functions (e.g., chemotaxis, immunomodulation, and hematopoietic stem cell homing and retention) [2,3]. CXCL12/CXCR4 axis is also involved in human pathological conditions, including virus infection, inflammatory/autoimmune diseases and cancer [4-6]. For example, CXCR4 is overexpressed in a plethora of hematopoietic and solid tumors and has been proposed as a biomarker for clinical prognosis [7,8]. Furthermore, CXCL12/CXCR4 axis contributes significantly to tumor metastasis [9]. Consequently, inhibition of the pathological functions of CXCL12/CXCR4 axis has been proposed as an important strategy for the development of drug candidates to treat HIV infection, inflammatory diseases and cancer.

Historically, the majority of the small molecule CXCR4 antagonists were intended for anti-HIV treatment. For example, AMD3100 (plerixafor, Figure 1) initially entered clinical trial as an anti-HIV agent [10]. However, poor oral bioavailability and dose-limiting adverse events made it unsuitable for the treatment of HIV infection [11]. Unexpected, AMD3100 exhibited strong mobilization effect on hematopoietic cells during clinical testing. Through further investigation, AMD3100 was subsequently approved by the FDA to treat patients with multiple myeloma and non-Hodgkin's lymphoma who have undergone stem cell transplantation [10]. This serendipitous discovery, which resulted in unprecedented therapeutic application, combined with the recent discovery of the multiple functions of CXCL12/CXCR4 axis in inflammatory disease and cancer, prompted significant expansion of efforts to pursue the next generation CXCR4 antagonists. The main challenge is to combine high potency with low toxicity and acceptable oral bioavailability. Towards this end, numerous small molecule CXCR4 antagonists had been synthesized, exemplified by AMD11070 [12-14], GSK812397 [15], TIQ15 [16], MSX-122 [17,18], KRH-3955 [19] and IT1t [20] (Figure 1). The majority of these molecules were synthesized in an effort to reduce the relatively high molecular weight and overall charge displayed by AMD3100. AMD11070 is the first oral CXCR4 antagonist that has been advanced to clinical investigation. Other compounds are in preclinical or early clinical testing.

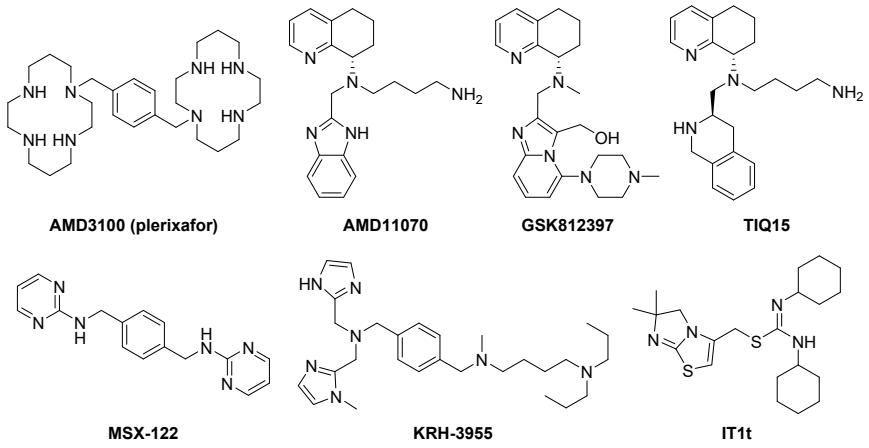


Fig. 1 Examples of small molecule CXCR4 antagonists reported in the literature.

We recently published a series of CXCR4 antagonists based on an aminopyrimidine scaffold as exemplified by compound **1** in Figure 2 [21,22]. In the process of structural optimization, we designed compound **2A** by introducing di-fluoro to mitigate metabolic liability of THQ ring (Scheme 1). In the process of the final substitution reaction, instead of **2A**, we accidentally synthesized compound **2**, the elimination product (Figure 2 and Scheme 1). Unexpectedly, compound **2** exhibited excellent binding affinity (inhibition of 12G5 competitive binding, IC_{50} = 15 nM) and functional activity (inhibition of CXCL12 induced calcium release, IC_{50} = 1.3 nM). These results were intriguing given that it had been well characterized that the chirality of the tetrahydroquinoline (THQ) ring was important for CXCR4 activity. Ordinarily, the S-enantiomer is at least 10 times more active than the R-enantiomer, and the configurational preference at this chiral center suggests that the orientation of the substituents is important for small molecule to interact with the CXCR4 receptor [13,14,21]. In contrast to the established structure-activity relationship, compound **2** demonstrated that replacement of the chiral center by an achiral double bond had no significant impact on activity. Following through on this observation, we synthesized the quinoline analogue **3** (Figure 2), which was also active (inhibition of 12G5 competitive binding, IC_{50} = 57 nM; inhibition of CXCL12 induced calcium release, IC_{50} = 0.24 nM). Elimination of the chiral center greatly simplifies the synthetic effort, which enables efficient SAR study. In addition, the moderate basicity combined with relative low molecular weight and clogP made compound **3** an attractive starting point for medicinal chemistry optimization. Here we report the SAR investigation based on compound **3** as a prototypical template.

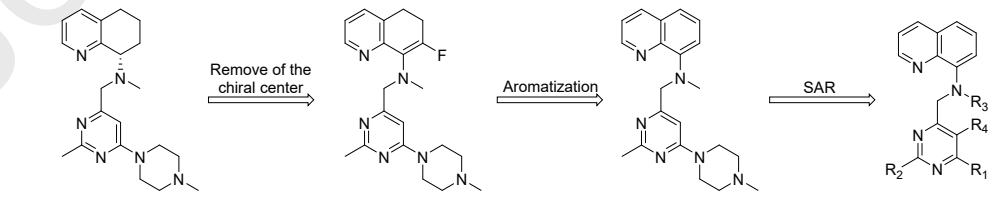
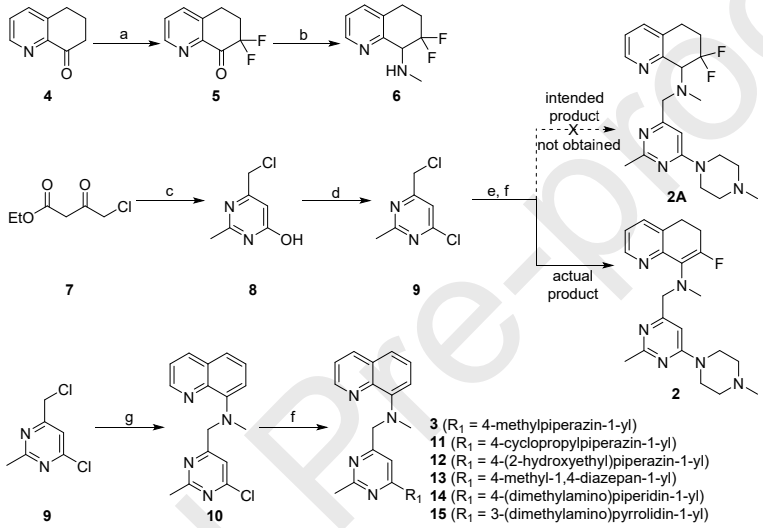


Fig. 2 Proposed SAR study plan.

2. Results and Discussion

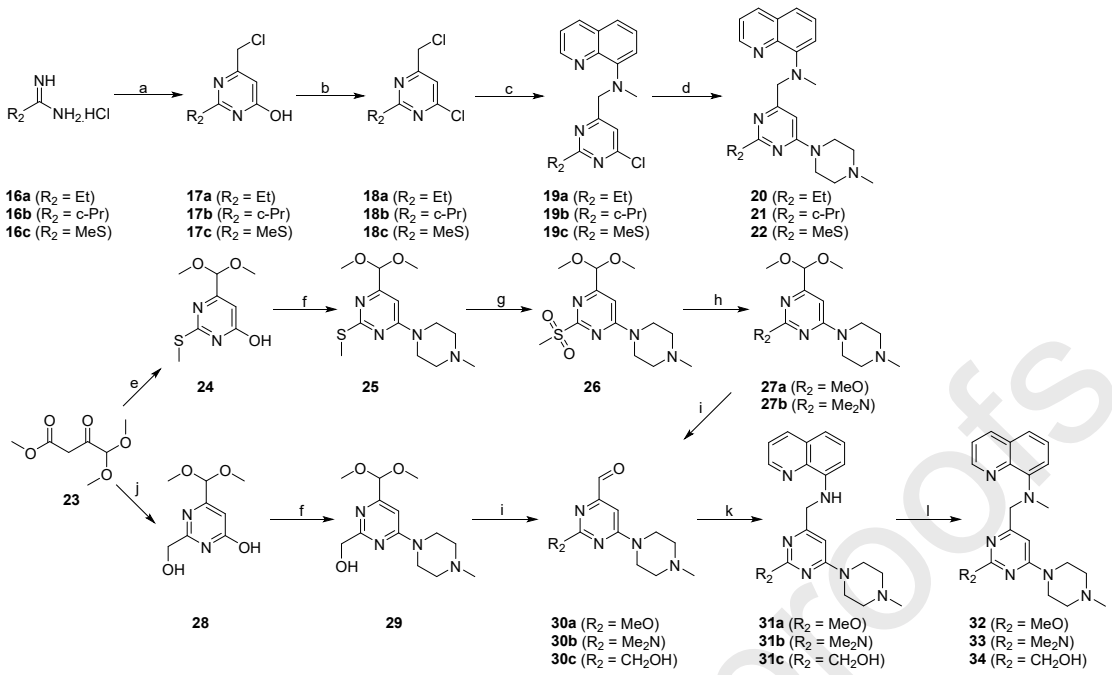
2.1. Chemistry

The synthesis of compounds **2**, **3** and **11-15** was performed as shown in Scheme 1. Fluorination of ketone **4** with selectfluor, followed by reductive amination with CH_3NH_2 yielded methylamine **6**. Cyclization of ester **7** with acetamidine hydrochloride, followed by chlorination with $POCl_3$ furnished intermediate **9**. Amine **6** was alkylated with intermediate **9**, subsequently substituted by *N*-methyl piperazine, and competitive elimination of hydrogen fluoride yielded compound **2** rather than the desired compound **2A**. Substitution of intermediate **9** with *N*-methylquinolin-8-amine gave intermediate **10**. The synthesis of compounds **3** and **11-15** was achieved by reacting **10** with diverse amines.



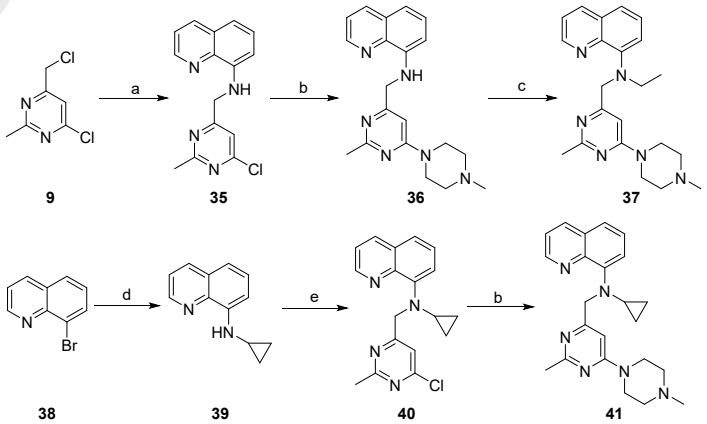
Scheme 1 Reagents and conditions: (a) NaH, THF, 0°C, 10 min, then selectfluor, rt, 1 h; (b) CH_3NH_2/EtOH, DCE, AcOH, DCE, $NaBH_3CN$, rt, overnight; (c) acetamidine hydrochloride, DBU, EtOH, 0 °C - rt, 12 h; (d) $POCl_3$, reflux, 1 h; (e) **6**, KI, DIPEA, MeCN, rt, overnight; (f) *N*-methyl piperazine, Et_3N, EtOH, reflux, overnight; (g) *N*-methylquinolin-8-amine, K_2CO_3, KI, MeCN, reflux, overnight; (h) corresponding amine, Et_3N, EtOH, reflux, overnight.

The general synthetic route used to prepare compounds **20-22 and 32-34** was outlined in Scheme 2. Chlorides **18a-c** were prepared from the corresponding amidines **16a-c** in two steps as the same route as intermediate **9**. Compounds **20-22** were synthesized from **18a-c** by substitution by *N*-methylquinolin-8-amine and *N*-methyl piperazine in sequence. Ester **23** [23] was reacted with 2-methyl-2-thiopseudourea sulfate or 2-hydroxyacetimidamide hydrochloride to provide pyrimidine **24** and **28**, followed by condensation with *N*-methyl piperazine in the presence of PyBOP to give intermediates **25** and **29**. Oxidation of **25** with Oxone followed by substitution with corresponding MeONa or dimethylamine afforded intermediates **27a** and **27b**. Aldehyde compounds **30a-c** were synthesized by deprotection of acetals **27a**, **27b** and **29**. Compounds **32-34** were obtained from aldehydes **30a-c** by reductive amination with quinolin-8-amine and subsequently with formaldehyde.



Scheme 2 Reagents and conditions: (a) **7**, DBU, EtOH, 0 °C - rt, 12 h; (b) $POCl_3$, reflux, 1 h; (c) *N*-methylquinolin-8-amine, K_2CO_3, KI, MeCN, reflux, overnight; (d) *N*-methyl piperazine, Et_3N, EtOH, reflux, overnight; (e) 2-methyl-2-thiopseudourea sulfate; K_2CO_3, H_2O, rt, overnight; (f) *N*-methyl piperazine, PyBOP, Et_3N, MeCN, reflux, overnight; (g) Oxone, THF, H_2O, rt, 4 h; (h) for **27a**: MeONa, MeOH, reflux, overnight; for **27b**: dimethylamine, THF, reflux, overnight; (i) 20% H_2SO_4, reflux, overnight; (j) 2-hydroxyacetimidamide hydrochloride, K_2CO_3, H_2O, rt, overnight; (k) quinolin-8-amine, $NaBH_3CN$, AcOH, MeOH, rt, overnight; (l) $HCHO/H_2O$, $NaBH_3CN$, AcOH, MeOH, rt, overnight.

Compounds **37** and **41** were prepared as described in Scheme 3. Substitution of chloride **9** with quinolin-8-amine and *N*-methyl piperazine in sequence gave intermediate **36**. Reductive amination of **36** with acetaldehyde yielded compound **37**. Compound **39** was formed from **38** by Buchwald coupling reaction with cyclopropylamine in the presence of $Pd_2(dba)_3$ and BINAP. Substitution of **9** with intermediate **39** gave intermediate **40**, which was reacted with *N*-methyl piperazine to afford compound **41**.

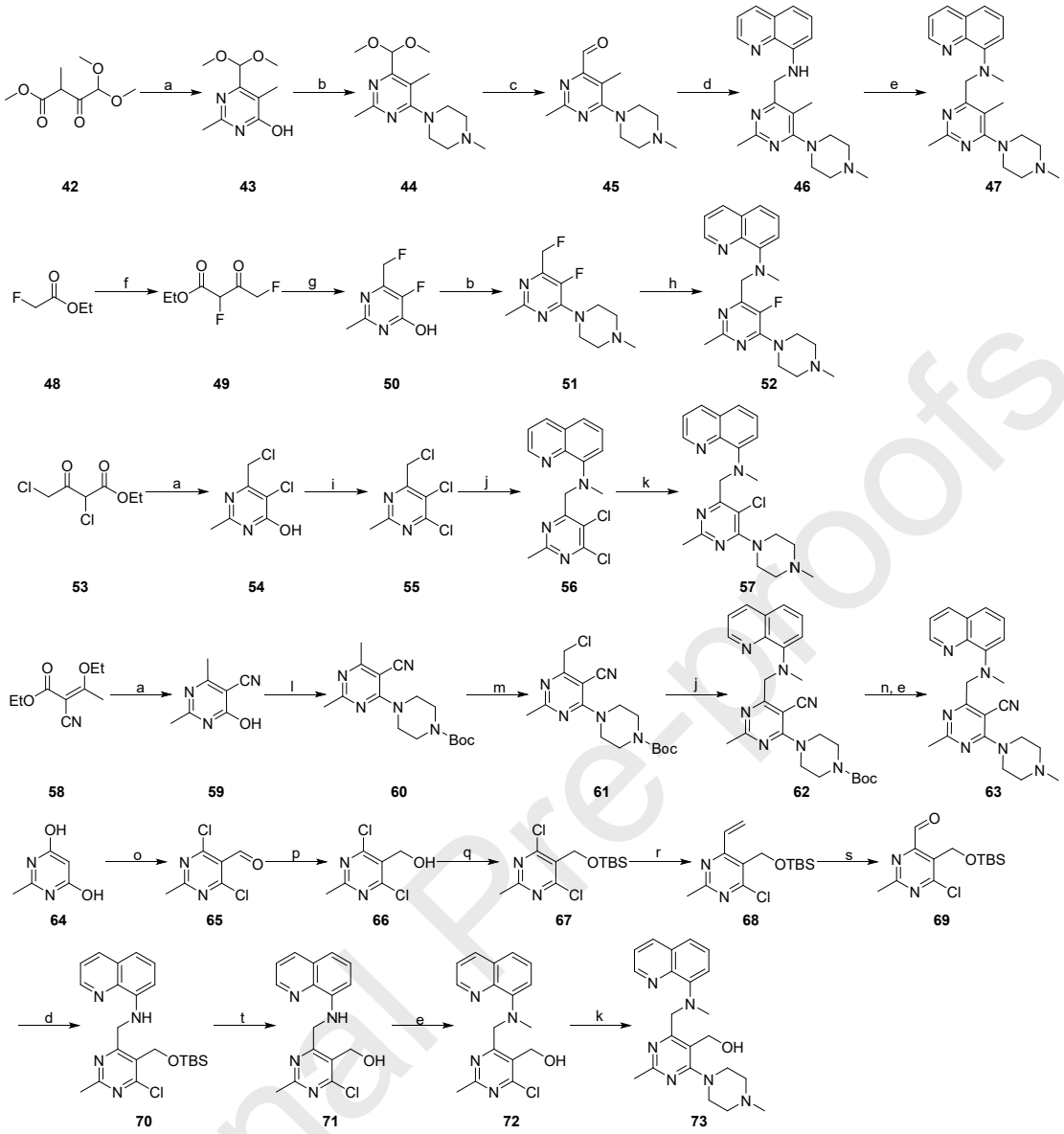


Scheme 3 Reagents and conditions: (a) quinolin-8-amine, K_2CO_3, KI, MeCN, reflux, overnight; (b) *N*-

methyl piperazine, Et_3N, EtOH, reflux, overnight; (c) acetaldehyde, $NaBH_3CN$, AcOH, MeOH, rt, overnight; (d) cyclopropanamine, $Pd_2(dba)_3$, BINAP, Cs_2CO_3, PhMe, 100 ^oC, overnight; (e) **9**, K_2CO_3, KI, MeCN, reflux, overnight.

The synthetic routes of compounds **47, 52, 57, 63 and 73** are shown in Scheme 4. Compound **47** was obtained from **42 [**23] by using the same route as compound **34**. The Claisen condensation reaction of ester **48** was used to get the intermediate **50**. Cyclization of **50** with acetimidamide hydrochloride gave intermediate **51**, which was substituted by *N*-methyl piperazine to provide compound **52**. Compound **57** can be prepared from **53 [**24] by using the same method as compound **3**. Cyclization of **58** with acetimidamide hydrochloride, followed by condensation with *N*-Boc-piperazine in the presence of PyBOP provided intermediate **60**. Trichloroisocyanuric acid was used to convert **60** to chloride **61**, which was substituted with *N*-methylquinolin-8-amine to generate intermediate **62**. Removal of the Boc group of **62** to get amine, followed by reductive amination with formaldehyde furnished compound **63**. Compound **73** could be synthesized in nine steps from 2-methylpyrimidine-4,6-diol **64**. The Vilsmeier-Haack reaction of **64** afforded aldehyde **65**, followed by reduction to alcohol with $NaBH_4$. Protection of the alcohol group with TBS followed by Suzuki–Miyaura reaction with potassium vinyltrifluoroborate furnished **68**. Oxidation of the double bond to aldehyde **69** with ozone, followed by the reductive amination with quinolin-8-amine led to intermediate **70**. Desilylation of **70** and reductive amination with formaldehyde gave intermediate **72**. Finally, treatment of **72** with *N*-methyl piperazine afforded compound **73**.

All final compounds were characterized by LC-MS, ^1H NMR, ^{13}C NMR and HRMS with at least 95% purity. The results of spectral analyses can be found in Supplementary Information.



Scheme 4 Reagents and conditions: (a) acetamidine hydrochloride, K_2CO_3, H_2O, rt, overnight; (b) *N*-methyl piperazine, PyBOP, Et_3N, MeCN, reflux, overnight; (c) 20% H_2SO_4, reflux, overnight; (d) quinolin-8-amine, $NaBH_3CN$, AcOH, MeOH, rt, overnight; (e) HCHO/H_2O, $NaBH_3CN$, AcOH, MeOH, rt, overnight; (f) NaH, Et_2O, reflux, 4 h; (g) acetamidine hydrochloride, EtONa, EtOH, reflux, overnight; (h) *N*-methylquinolin-8-amine, 2-ethyl-2-(hydroxymethyl)propane-1,3-diol, KI, H_2O, reflux, overnight; (i) $POCl_3$, reflux, 1 h; (j) *N*-methylquinolin-8-amine, K_2CO_3, KI, MeCN, reflux, overnight; (k) *N*-methyl piperazine, Et_3N, EtOH, reflux, overnight; (l) *N*-Boc-piperazine, PyBOP, Et_3N, MeCN, reflux, overnight; (m) trichloroisocyanuric acid, CH_2Cl_2, rt, 6 h; (n) TFA, CH_2Cl_2, rt, 2 h; (o) $POCl_3$, DMF, 0 ^oC, 1h; reflux, overnight; (p) $NaBH_4$, THF, H_2O, 0 ^oC, 30 min; (q) TBSCl, imidazole, CH_2Cl_2, rt, overnight; (r) potassium vinyltrifluoroborate, $Pd(PPh_3)_4$, Cs_2CO_3, dioxane, H_2O, 100 ^oC, overnight; (s) ozone, MeOH, CH_2Cl_2, -65 ^oC, 1 h; Me_2S, -65 ^oC, 10 min; (t) pyridine hydrofluoride, THF, rt, 4 h.

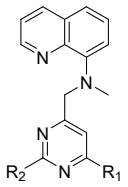
2.2. Structure-activity relationship based on calcium mobilization assay

Numerous assays have been used to characterize the CXCL12/CXCR4 axis. The reported assays include competitive binding assay, CXCL12-induced calcium mobilization, CXCR4 receptor

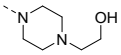
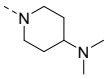
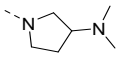
internalization, CXCL12-guided chemotaxis and CXCR4-specific HIV entry and replication. In our previous publications, we used a cell based FACS 12G5 competitive binding assay as the primary screening assay followed by a FLIPR Tetra based cytosolic calcium flux assay as the functional assay [21]. In this report, we used FLIPR Tetra based cytosolic calcium flux assay [21,25] as our primary screening assay followed by the 12G5 competitive binding assay [21,26] as the on-target assay. Competitive ligand binding assays are good for characterization of target engagement but convey no functional information. On the other hand, calcium flux assay provides information regarding receptor activation, inhibition, or potentiation. In addition, competitive ligand binding assays tend to miss allosteric binders which do not compete for the same binding pocket. More importantly, based on our experience, the results from the 12G5 competitive binding assay correlate very well with the results from the functional calcium flux assay. Furthermore, the expense of the calcium flux assay was lower and the throughput was higher compared with the 12G5 competitive binding assay. Based on these considerations, we decided to use the FLIPR Tetra based calcium flux functional assay as the screening assay and the 12G5 competitive binding assay as the confirmation assay. The structure-activity-relationship is summarized in Tables 1 and 2.

We started structural modifications on the R_1 position, which is solvent exposed according to our previous docking study. We used a small set of basic amines to test if the SAR on R_1 would follow the established trend. Replacing the methyl group on the terminal nitrogen of the piperazine by a larger cyclopropyl group had a negative effect, leading to a 7-fold decrease of potency (1.5 nM vs. 0.24 nM for compounds **11** and **3**, respectively). Similarly, introducing a polar hydroxyl group resulted in a 10-fold decrease of potency (compound **12**, 2.1 nM). Ring expansion (compound **13**, 0.81 nM), extension of the nitrogen out of the piperazine ring (compound **14**, 2.3 nM), and introduction of amino pyrrolidine (compound **15**, 0.71 nM) to increase pKa (8.8-9.0, Table 1) all led to decreased potency. The SAR on the basic R_1 position largely followed our previous SAR results [21,22]. With piperazine fixed on R_1, we next explored R_2 with different substituents. Increase size on R_2 led to decreased potency (compounds **20** and **21**; 0.73 and 2.9 nM, respectively). Introduction of heteroatoms have distinct impact: while methylthio and methoxy were tolerated (compound **22** and **23**, 0.57 and 3.0 nM, respectively), the dimethylamino led to significant decrease of potency (compound **33**, 1926 nM). Reduction of clogP by incorporation of a hydroxymethyl group resulted in 30-fold decrease of potency (compound **34**, 7.3 nM).

Table 1. SAR for R_1 and R_2



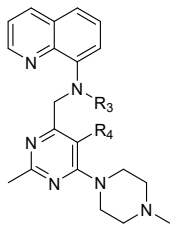
Cmpd	R_1	R_2	Ca^{2+} Flux IC_{50} (nM) [a]	clogP[b]	pKa[c]	Cmpd	R_1	R_2	Ca^{2+} Flux IC_{50} (nM) [a]	clogP[b]	pKa[c]
3		Me	0.24 ± 0.01	2.13	7.0	**20**		Et	0.73 ± 0.48	2.71	7.1
11		Me	1.5 ± 0.04	2.50	7.0	**21**			2.9 ± 0.03	2.50	7.2

12		Me	2.1 ± 0.005	1.50	7.0	**22**		MeS	0.57 ± 0.06	3.52	6.3
13		Me	0.81 ± 0.47	2.40	8.8	**32**		MeO	3.0 ± 0.6	2.42	6.3
14		Me	2.3 ± 0.5	2.66	9.0	**33**		Me_2N	1926 ± 470	3.00	8.8
15		Me	0.76 ± 0.08	2.39	8.8	**34**		$HOCH_2$	7.3 ± 0.7	1.49	5.9
AMD310 0			23 ± 2.3	-1.04	10.6						

[a] Inhibition of luminescence signaling in FLIPR Tetra calcium mobilization 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.

Having established piperazine and methyl as the preferred substituents on R_1 and R_2 positions respectively, we set to focus on the SAR of R_3 and R_4 positions. Replacing the methyl with an ethyl on R_3 led to similar activity (compound **37**, 0.16 nM) to compound **3**. Further increase on size led to decreased potency (compound **41**, 2.5 nM). R_4 position had been used to introduce polar functional groups as a way to fine tune physicochemical properties [27,28]. The synthesis of R_4 analogues was challenging because R_4 was difficult to be modified at the final steps. As a result, each of the five analogues was synthesized by a different synthetic route, albeit in a similar fashion. Replacing the hydrogen with a methyl or a fluorine led to compounds with similar activity (compounds **47** and **52**, 0.49 and 0.86 nM, respectively) to compound **3**. Chlorine, cyano and hydroxymethyl group were all tolerated but showed decrease of potency to various extents (compounds **57**, **63**, and **73**; 1.2, 3.5, 0.95 nM, respectively). Overall, the SAR of the aforementioned locations generally followed the established SAR on the tetrahydroquinoline series. The most intriguing result was the lack of impact on potency with the elimination of the chiral center on the tetrahydroquinoline scaffold.

Table 2. SAR for R_3 and R_4



Cmpd	R_3	R_4	Ca^{2+} flux IC_{50} (nM) [a]	clogP[b]	pKa[c]
3	Me	H	0.24 ± 0.01	2.13	7.0
37	Et	H	0.16 ± 0.01	2.51	8.1
41		H	2.5 ± 0.9	2.50	7.2
47	Me	Me	0.49 ± 0.08	2.51	7.4
52	Me	F	0.86 ± 0.18	2.22	5.9
57	Me	Cl	1.2 ± 0.3	2.74	5.6
63	Me	CN	3.5 ± 0.4	1.81	5.2
73	Me	CH_2OH	0.95 ± 0.04	1.40	6.7

AMD3100	23 ± 2.3	-1.04	10.6

[a] Inhibition of luminescence signaling in FLIPR Tetra calcium mobilization 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.

2.3. Binding assay and preliminary selectivity assays

Compounds **3** and **37** emerged as the leads of this aminoquinoline series of analogues based on their excellent functional activity and overall properties (e.g., compound **3**, MW 362, clogP 2.1, PSA 48, pKa 7.0). We next tested compounds **3** and **37** in the 12G5 competitive binding assay to see if they bind with the CXCR4 receptor. In this assay, HPB-ALL cells which naturally express CXCR4 were incubated with APC-conjugate clone 12G5 antibody and testing compound. The inhibition of the 12G5 antibody signal with different concentrations of compound provided inhibition curve and readout of IC_{50}. The detailed description of assay development and validation had been reported [21,26]. Compounds **3** and **37** potently replaced 12G5 antibody at IC_{50} = 57 ± 8.0 nM and 144 ± 7.3 nM, respectively; while the positive control AMD3100 replaced the 12G5 antibody at IC_{50} = 561 ± 27 nM. Representative single assay curves were shown in Figure 3. These data established compounds **3** and **37** as competitive CXCR4 antagonists.

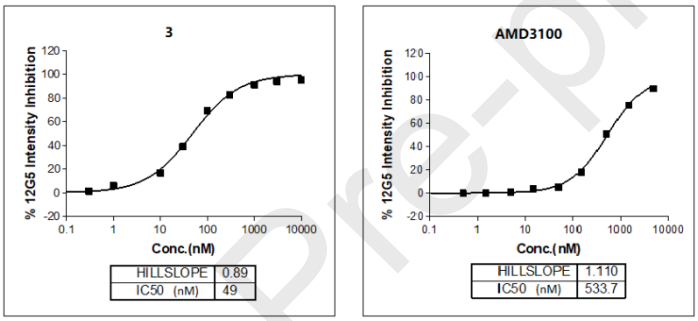


Fig. 3 Dose response curves of compound **3** and AMD3100 in the 12G5 HPB-ALL CXCR4 competitive binding assay.

Having demonstrated that compound **3** potently binds to CXCR4 (IC_{50} = 57 nM) and inhibits CXCL12 induced cytosolic calcium flux (IC_{50} = 0.24 nM), we further assessed the preliminary functional specificities of compound **3**. We chose CXCR1 as a representative within the CXCR sub-family and CCR6 as a representative outside of the CXCR sub-family and tested compound **3** in the corresponding functional assays. Compound **3** was shown to be inactive up to 10,000 nM (Figure 4b, c).

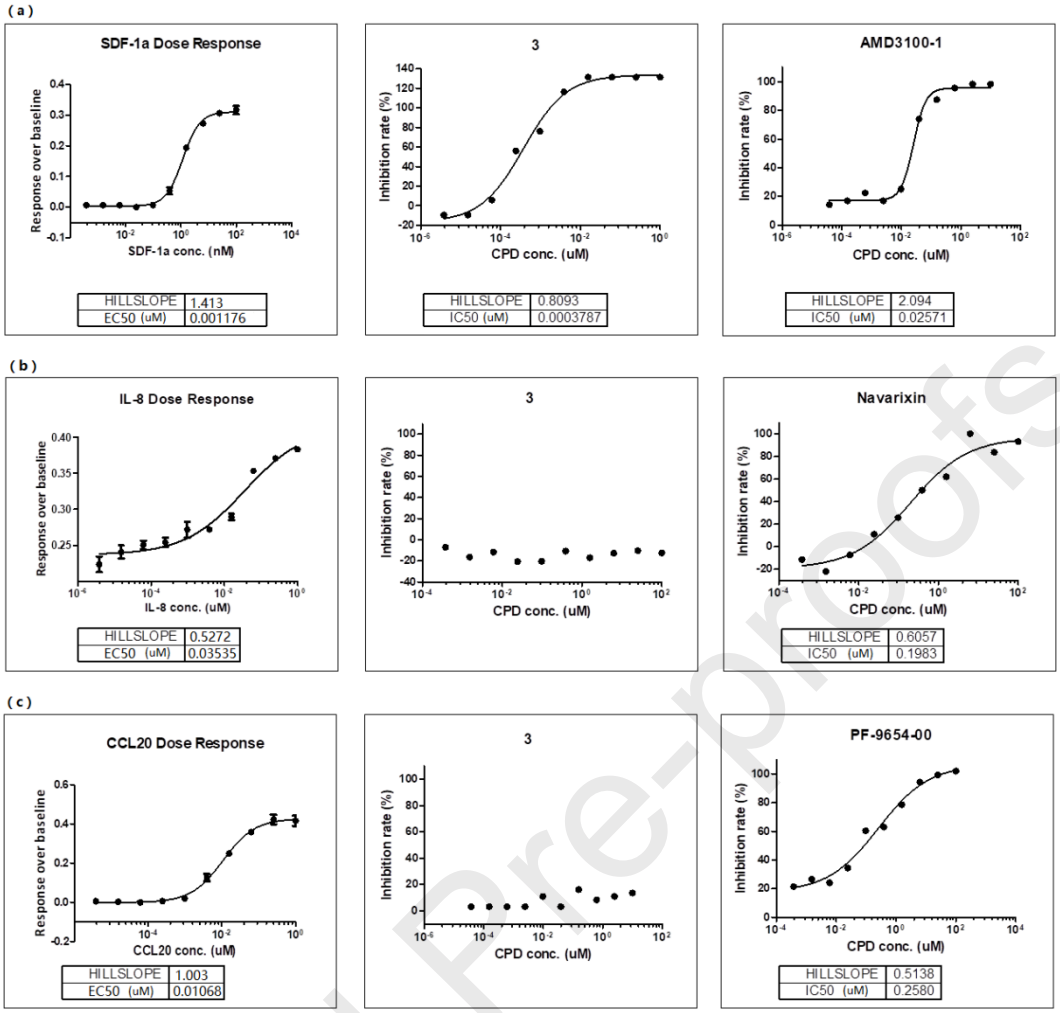


Fig. 4 Compound **3** is a potent CXCR4 antagonist, but is inactive to CXCR1 and CCR6. (a) CXCR4 dose response curve for SDF-1 (CXCL12) stimulation and inhibition curves of compound **3**/AMD3100 (positive control for CXCR4) based on a calcium flux assay. (b) CXCR1 dose response curve for IL-8 stimulation and inhibition curves of compound **3**/Navarixin (positive control for CXCR1) based on a functional cAMP assay. (c) CCR6 dose response curve for CCL20 stimulation and inhibition curves of compound **3**/PF-9654-00 (positive control for CCR6) based on a calcium flux assay.

2.4. Chemotaxi assay based on transwell and cell toxicity assays

CXCR4 is a chemokine receptor. One of the hallmarks of CXCR4 activation by CXCL12 is the migration and invasion of the corresponding cells. We investigated compound **3** in the matrigel invasion assay to evaluate its inhibition of CXCL12/CXCR4 mediated chemotaxis and invasion. To the upper chamber were added compound **3** or AMD3100 (100 nM) and CXCR4 expressing MDA-MB-231 cells. To the lower chamber was added human CXCL12 (200 ng/mL). The inhibition of cell invasion with compound **3** or AMD3100 was calculated by comparison of the cell invasion without compound treatment (Figure 5a, b). Compound **3** inhibited cell migration more potently in the matrigel invasion assay than AMD3100, consistent with the calcium flux and the 12G5 binding assays. Cell mobility can be affected by compounds with general cellular toxicity. To rule out this possibility, we used cell viability assay (ATP-based Cell Titer-Glo Luminescent Cell Viability Assay) to investigate the potential cytotoxicity effect of compound **3**. As shown in Figure 5c, compound **3** did not inhibit cell proliferation

of multiple human and murine cells at concentrations up to 1000 nM.

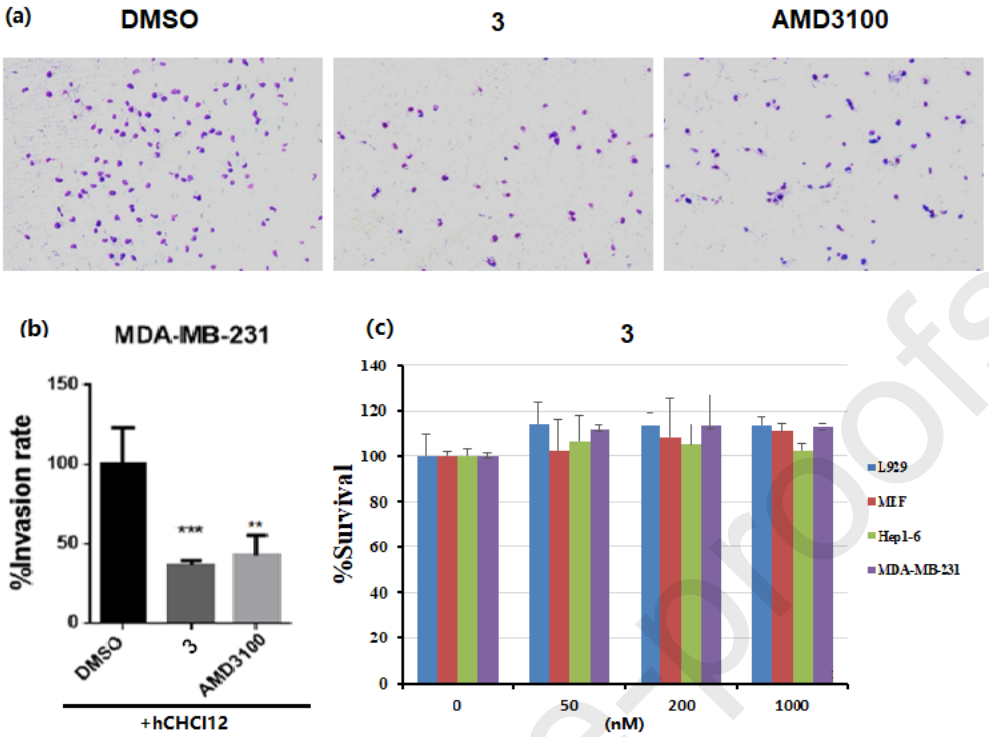


Fig. 5 Effect of compound **3** on matrigel invasion of MDA-MB-231 cells and cytotoxicity assessment of **3** in the cultured human and murine cells. (a) Photo images of matrigel 22 h after invasion experiment. (b) Quantification of transwell analysis of cell invasion experiment. Data represent mean value ± standard deviation. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Magnification: 4x. (c) Effect of compound **3** on proliferation of human and murine cells.

2.5. Preliminary in vitro safety and DMPK evaluation

AMD11070 was derived from AMD3100 to reduce molecular weight and over charges. As a result, AMD11070 can be administered orally and has been progressed into clinical testing. However, CYP isozyme (3A4, 2D6) inhibition was reported and the possible culprit had been attributed to the benzimidazole functionality [14,21]. In our previous work, we used aminopyrimidine to replace the benzimidazole as a way to mitigate CYP inhibition. In order to understand the CYP inhibition profile of this new aminoquinoline template, we tested compound **3** in CYP isozyme inhibition assays. As shown in Table 3, compound **3** exhibited minimal inhibition of CYP3A4 and 2D6 at 10 μM concentration, while AMD11070 demonstrated moderate inhibition (60% and 64%; 3A4 and 2D6, respectively) [21]. Compound **3** was found to be highly permeable (45× 10^{-6} cm/s, Caco-2), and not likely to be an efflux substrate (Table 3). Moreover, compound **3** exhibited high plasma protein binding in human (99%) but more moderate binding in mouse and rat (94% and 90%, respectively). However, despite the marginally improved metabolic stability in human liver microsomes, compound **3** was quickly metabolized in rat liver microsomes (Table 3). Improvement of metabolic stability cross species must be considered in the future medicinal chemistry optimization.

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

Cmpd	CYP Inhibition	Caco-2[a]	PPB (%)[b]	RLM[c]	HLM[d]

	(%)[a]										
	3A4	2D6	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)	Cl_{int} (mL/min/kg)	$t_{1/2}$ (min)
3	13	15	45	0.52	94	90	99	656	3.8	122	14.2

[a] The compound was tested at 10 μM concentration. [b] The compound was tested at 1 μM concentration. [c] RLM = Rat liver microsomes. [d] HLM = Human liver microsomes.

3. Conclusion

An aminoquinoline template was used to develop a novel series of CXCR4 antagonists. This template is devoid of the chiral center in the classical tetrahydroquinoline (THQ) ring moiety and therefore can be easily synthesized. A number of potent CXCR4 antagonists were identified, exemplified by compound **3**, which demonstrated excellent binding affinity with CXCR4 receptor (IC_{50} = 57 nM) and inhibited CXCL12 induced cytosolic calcium increase (IC_{50} = 0.24 nM). Furthermore, compound **3** potently inhibited CXLC12/CXCR4 mediated cell migration in a transwell invasion assay. The simplified synthetic approach combined with good physicochemical properties (e.g. MW 362, clogP 2.1, PSA 48, pKa 7.0 for compound **3**) demonstrate the potential of this aminoquinoline template as a novel scaffold to develop CXCR4 antagonists. Structural modification on the aminoquinoline is ongoing and the results will be reported in due course.

4. Experimental protocols

4.1. Chemistry

All reagents and solvents were used without 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. HPLC method: Waters Acquity UPLC, BEH C18 2.1 mm × 50 mm, 1.7 μm particles. Mobile phase A: 5 mM aqueous ammonium acetate. Mobile phase B: MeOH. Temperature: 24 °C. Gradient: 5−40% B over 1 min, 40-70% B over 1 min, 70-95% B over 4 min, then a 1 min hold at 95% B. Flow: 1.2 mL/min. Detection: UV at 214 and 254 nm. The detailed procedures of chemical experiment can be found in the supporting information.

*4.1.1. 7,7-Difluoro-6,7-dihydroquinolin-8(5H)-one (**5**)*

To a solution of NaH (60 wt percent moistened with oil, 511 mg, 12.8 mmol) in THF (20 mL) was added dropwise the THF solution (5 mL) containing compound **4** (588 mg, 4 mmol) at 0°C. The reaction was stirred at 0°C for 10 min followed by adding selectfluro (3.0 g, 8.4 mmol) in portions. The reaction mixture was stirred at room temperature for 1h. Water (20 mL) was added to quench the reaction. The water phase was extracted with diethyl ether (20 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 (petroleum ether/ethyl acetate = 10/1) to give the desired product (380 mg, 52%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (d, J = 4.4 Hz, 1H), 7.73 (d, J = 7.6 Hz, 1H), 7.55-7.45 (m, 1H), 3.20 (t, J = 6.4 Hz, 1H), 2.72-2.56 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 183.9 (t, J = 26.3 Hz), 150.4, 145.9, 139.8, 137.8, 128.2, 113.8 (t, J = 247.8 Hz), 32.0 (t, J = 23.0 Hz), 24.4 (t, J = 5.9 Hz). MS (ESI/APCI)

m/z 183.9 $[M+H]^+$.

*4.1.2. 7,7-Difluoro-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**6**)*

To a solution of **5** (18.3 mg, 0.1 mmol), $MeNH_2$ solution (30 wt percent in ethanol, 1 mL) and HOAc (5 mg, 0.083 mmol) in 1,2-dichloroethane (2 mL) was added $NaBH_3CN$ (13 mg, 0.2 mmol). The resulting suspension was stirred at room temperature overnight. The reaction mixture was filtered and the filtrate was evaporated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 150/1) to give the desired product (15 mg, 75%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J* = 4.4 Hz, 1H), 7.47 (d, *J* = 7.6 Hz, 1H), 7.21-7.14 (m, 1H), 3.89 (t, *J* = 10.0 Hz, 1H), 3.01-2.92 (m, 2H), 2.72 (s, 3H), 2.57-2.44 (m, 1H), 2.28-2.18 (m, 1H). MS (ESI/APCI) *m/z* 199.0 $[M+H]^+$.

*4.1.3. 6-(Chloromethyl)-2-methylpyrimidin-4-ol (**8**)*

To a solution of ethyl 4-chloro-3-oxobutanoate **7** (22 g, 136 mmol) and acetimidamide hydrochloride (17 g, 150 mmol) in ethanol (180 mL) was slowly added DBU (41 g, 272 mmol) at 0 °C. The reaction was stirred at room temperature for 12 h. The reaction solution was evaporated. 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 silica gel column chromatography (petroleum ether/ethyl acetate = 2.5/1) to give the desired product (8 g, 37%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.54 (br s, 1H), 6.29 (s, 1H), 4.44 (s, 2H), 2.28 (s, 3H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 162.4, 161.8, 159.8, 110.4, 45.3, 21.1. MS (ESI/APCI) *m/z* 156.9 $[M-H]^-$.

*4.1.4. 4-Chloro-6-(chloromethyl)-2-methylpyrimidine (**9**)*

The solution of **8** (8.0 mmol, 50 mmol) in $POCl_3$ (40 mL) was stirred at reflux for 1 h. The reaction mixture was concentrated and dissolved in ethyl acetate (200 mL), then added saturated $NaHCO_3$ solution to adjust pH to 7, extracted with ethyl acetate (100 mL * 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 give the desired product (5.2 g, 59%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (s, 1H), 4.55 (s, 2H), 2.70 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.1, 166.7, 162.2, 116.7, 44.7, 25.8. MS (ESI/APCI) *m/z* 176.9 $[M+H]^+$.

*4.1.5. 7-Fluoro-N-methyl-N-((2-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-5,6-dihydroquinolin-8-amine (**2**)*

A mixture of **6** (55 mg, 0.28 mmol), **9** (54 mg, 0.31 mmol), KI (5 mg, 0.028 mmol) and DIPEA (90 mg, 0.7 mmol) in CH_3CN (10 mL) was stirred at room temperature overnight. The reaction solution was evaporated to remove most of CH_3CN, diluted with dichloromethane (15 mL) and washed with saturated brine solution (10 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was dissolved in ethanol (10 mL). TEA (252 mg, 2.5 mmol) and *N*-methylpiperazine (125 mg, 1.25 mmol) were added and stirred at reflux overnight. The reaction mixture was cooled to room temperature and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/methanol/ammonium hydroxide = 50/1/1) to give the desired product (15 mg, 15%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, *J* = 4.0 Hz, 1H), 7.33(d, *J* = 7.2 Hz, 1H), 7.04-6.90 (m, 2H), 4.26 (s, 2H), 3.68-3.58 (m, 4H), 2.95-2.80 (m, 5H), 2.61-2.53 (m, 2H), 2.47 (s, 3H), 2.45-2.39 (s, 4H), 2.32 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.1, 166.4, 162.8, 158.8, 157.0, 152.8 (d, *J* = 9.6 Hz), 147.3, 134.5, 129.7, 127.0 (d, *J* = 7.1 Hz), 121.0, 97.4, 59.5, 54.7, 46.1, 43.7, 41.9 (d, *J* = 4.2 Hz), 27.0 (d, *J* = 8.0 Hz), 25.8, 25.1 (d, *J* = 23.4 Hz). HRMS (ESI): calcd for $C_{21}H_{28}FN_6$ $[M+H]^+$ 383.2354, found 383.2365. Purity: 98.8%.

*4.1.6. N-((6-Chloro-2-methylpyrimidin-4-yl)methyl)-N-methylquinolin-8-amine (**10**)*

A mixture of **9** (890 mg, 5.0 mmol), *N*-methylquinolin-8-amine (530 mg, 3.4 mmol), KI (56 mg, 0.34 mmol) and K_2CO_3 (925 mg, 6.7 mmol) in CH_3CN (20 mL) was stirred at reflux overnight. The reaction solution was evaporated to remove most of CH_3CN. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 100/1) to give the desired product (1.04 g, 98%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (d, *J* = 2.4 Hz, 1H), 8.13 (d, *J* = 8.4 Hz, 1H), 7.67 (s, 1H), 7.48-7.34 (m, 3H), 7.14 (d, *J* = 7.6 Hz, 1H), 4.83 (s, 2H), 3.04 (s, 3H), 2.72 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.9, 168.4, 161.4, 148.1, 147.7, 142.0, 136.9, 129.9, 126.9, 121.2, 120.8, 117.1, 115.7, 61.7, 41.1, 25.9. MS (ESI/APCI) *m/z* 298.8 $[M+H]^+$.

*4.1.7. General procedure for the synthesis of **3** and **11-15***

A mixture of **10** (0.17 mmol, 1.0 eq.), TEA (10.0 eq.) and corresponding amine (5.0 eq.) in ethanol (5 mL) was stirred at reflux overnight. The reaction solution was evaporated. The residue was purified by silica gel column chromatography (dichloromethane/methanol/ammonium hydroxide = 50/1/1) to give the desired product.

*4.1.7.1. N-Methyl-N-((2-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)quinolin-8-amine (**3**)*

Compound **3** was obtained as a yellow oil (yield 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (d, *J* = 2.4 Hz, 1H), 8.11 (d, *J* = 8.4 Hz, 1H), 7.44-7.35 (m, 3H), 7.12 (d, *J* = 7.2 Hz, 1H), 6.67 (s, 1H), 4.68 (s, 2H), 3.56 (s, 4H), 3.05 (s, 3H), 2.51 (s, 3H), 2.42-2.36 (m, 4H), 2.29 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.1, 166.8, 162.8, 148.9, 147.8, 142.7, 136.5, 129.9, 126.8, 121.0, 120.6, 116.2, 97.9, 61.8, 54.8, 46.3, 43.8, 41.5, 26.2. HRMS (ESI): calcd for $C_{21}H_{27}N_6$ $[M+H]^+$ 363.2292, found 363.2296. Purity: 96.9%.

*4.1.7.2. N-((6-(4-Cyclopropylpiperazin-1-yl)-2-methylpyrimidin-4-yl)methyl)-N-methylquinolin-8-amine (**11**)*

Compound **11** was obtained as a yellow oil (yield 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (s, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 7.46-7.30 (m, 3H), 7.11 (d, *J* = 7.2 Hz, 1H), 6.67 (s, 1H), 4.69 (s, 2H), 3.59-3.40 (m, 4H), 3.05 (s, 3H), 2.63-2.54 (m, 4H), 2.51 (s, 3H), 1.67-1.51 (m, 1H), 0.53-0.33 (m, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.7, 166.6, 162.6, 148.9, 147.7, 142.6, 136.5, 129.8, 126.7, 121.0, 120.6, 116.2, 97.9, 61.6, 53.0, 43.8, 41.5, 38.5, 26.1, 5.9. HRMS (ESI): calcd for $C_{23}H_{29}N_6$ $[M+H]^+$ 389.2448, found 389.2454. Purity: 98.0%.

*4.1.7.3. 2-(4-(2-Methyl-6-((methyl(quinolin-8-yl)amino)methyl)pyrimidin-4-yl)piperazin-1-yl)ethan-1-ol (**12**)*

Compound **12** was obtained as a yellow oil (yield 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (d, *J* = 4.4 Hz, 1H), 8.08 (d, *J* = 8.0 Hz, 1H), 7.43-7.29 (m, 3H), 7.11 (d, *J* = 7.2 Hz, 1H), 6.66 (s, 1H), 4.65(s, 2H), 3.66-3.59 (m, 2H), 3.58-3.48 (m, 4H), 3.03 (s, 3H), 2.64-2.41 (m, 10H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.1, 166.7, 162.6, 148.8, 147.7, 142.6, 136.5, 129.8, 126.7, 121.0, 120.6, 116.1, 97.8, 61.6, 59.6, 57.9, 52.6, 43.8, 41.5, 26.1. HRMS (ESI): calcd for $C_{22}H_{29}N_6O$ $[M+H]^+$ 393.2397, found 393.2399. Purity: 95.7%.

*4.1.7.4. N-Methyl-N-((2-methyl-6-(4-methyl-1,4-diazepan-1-yl)pyrimidin-4-yl)methyl)quinolin-8-amine (**13**)*

Compound **13** was obtained as a yellow oil (yield 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (d, *J* = 2.4 Hz, 1H), 8.10 (d, *J* = 8.0 Hz, 1H), 7.45-7.32 (m, 3H), 7.11 (d, *J* = 7.2 Hz, 1H), 6.44 (s, 1H), 4.72 (s, 2H), 4.05 -3.16 (m, 4H), 3.08 (s, 3H), 2.72-2.38(m, 7H), 2.30 (s, 3H), 1.78 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.5, 166.3, 162.1, 148.8, 147.7, 142.6, 136.4, 129.8, 126.7, 121.0, 120.5, 116.2, 97.4, 61.5, 58.0, 57.3, 46.7, 46.0, 45.5, 41.7, 27.2, 26.3. HRMS (ESI): calcd for $C_{22}H_{29}N_6$ $[M+H]^+$ 377.2448, found

377.2440. Purity: 95.5%.

*4.1.7.5. N-((6-(4-(Dimethylamino)piperidin-1-yl)-2-methylpyrimidin-4-yl)methyl)-N-methylquinolin-8-amine (**14**)*

Compound **14** was obtained as a yellow oil (yield 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88-8.76 (m, 1H), 8.11 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.46-7.32 (m, 3H), 7.12 (dd, *J* = 7.2, 2.0 Hz, 1H), 6.66 (s, 1H), 4.68 (s, 2H), 4.37(d, *J* = 13.2 Hz, 2H), 3.05 (s, 3H), 2.75 (t, *J* = 7.2 Hz,2H), 2.52-2.46 (m, 4H), 2.32 (s, 6H), 1.86 (d, *J* = 12.8 Hz, 2H), 1.43-1.33 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.0, 166.8, 162.4, 148.9, 147.8, 142.7, 136.5, 129.9, 126.8, 121.1, 120.7, 116.3, 97.9, 62.6, 61.7, 43.3, 41.6, 41.4, 27.7, 26.2. HRMS (ESI): calcd for $C_{23}H_{31}N_6$ $[M+H]^+$ 391.2605, found 391.2613. Purity: 97.8%.

*4.1.7.6. N-((6-(3-(Dimethylamino)pyrrolidin-1-yl)-2-methylpyrimidin-4-yl)methyl)-N-methylquinolin-8-amine (**15**)*

Compound **15** was obtained as a yellow oil (yield 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (d, *J* = 4.4 Hz,1H), 8.09 (d, *J* = 8.0 Hz,1H), 7.46-7.30 (m, 3H), 7.12 (d, *J* = 7.2 Hz, 1H), 6.34 (s, 1H), 4.74 (s, 2H), 4.15-3.14 (m, 4H), 3.08 (s, 3H), 2.68 (s, 1H), 2.53 (s, 3H), 2.25 (s, 6H), 2.18-2.05 (m, 1H), 1.80 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.5, 165.6, 160.5, 148.7, 147.5, 142.4, 136.3, 129.7, 126.6, 120.8, 120.4, 116.0, 98.1, 65.1, 61.3, 50.5, 45.4, 44.3, 41.4, 30.0, 26.0. HRMS (ESI): calcd for $C_{22}H_{29}N_6$ $[M+H]^+$ 377.2448, found 377.2456. Purity: 99.0%.

*4.1.8. General procedure for the synthesis of **17a-c***

To a solution of **16a**/**b**/**c** (9 mmol, 1.0 eq.) and **7** (1.0 eq.) in ethanol (15 mL) was slowly added DBU (2.0 eq.) at 0 °C. The reaction was stirred at room temperature for 12 h. The reaction solution was evaporated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 2.5/1) to give the desired product.

*4.1.8.1. 6-(Chloromethyl)-2-ethylpyrimidin-4-ol (**17a**)*

Compound **17a** was obtained as a yellow oil (yield 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.97 (s, 1H), 6.54 (s, 1H), 4.39 (s, 2H), 2.73 (q, *J* = 7.6 Hz, 2H), 1.35 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.0, 164.3, 163.7, 110.2, 45.3, 28.8, 11.7. MS (ESI/APCI) *m/z* 170.8 $[M-H]^-$.

*4.1.8.2. 6-(Chloromethyl)-2-cyclopropylpyrimidin-4-ol (**17b**)*

Compound **17b** was obtained as a yellow oil (yield 36%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.52 (s, 1H), 6.46 (s, 1H), 4.30 (s, 2H), 2.00-1.81 (m, 1H), 1.26-1.17 (m, 2H), 1.15-1.06 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.0, 164.7, 164.6, 108.9, 45.4, 14.5, 11.0. MS (ESI/APCI) *m/z* 184.9 $[M+H]^+$.

*4.1.8.3. 4-Chloro-6-(chloromethyl)-2-(methylthio)pyrimidine (**17c**)*

Compound **17c** was obtained as a yellow oil (yield 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.71 (s, 1H), 6.41 (s, 1H), 4.35 (s, 2H), 2.59 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.5, 163.4, 162.4, 108.3, 45.2, 13.4. MS (ESI/APCI) *m/z* 191.1 $[M+H]^+$.

*4.1.9. General procedure for the synthesis of **18a-c***

The solution of **17a**/**b**/**c** (4.1 mmol, 1.0 eq.) in $POCl_3$ (2.5 mL) was heated to 90 °C, and stirred for 1 h. Then reaction mixture was concentrated and dissolved in ethyl acetate (20 mL), then added saturated $NaHCO_3$ solution to adjust pH to 7, extracted with ethyl acetate (10 mL * 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 give the desired product.

*4.1.9.1. 4-Chloro-6-(chloromethyl)-2-ethylpyrimidine (**18a**)*

Compound **18a** was obtained as a yellow oil (yield 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (s, 1H), 4.57 (s, 2H), 2.96 (q, *J* = 7.6 Hz, 2H), 1.35 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.3, 166.7, 162.3, 116.8, 44.8, 32.5, 12.6. MS (ESI/APCI) *m/z* 190.9 $[M+H]^+$.

*4.1.9.2. 4-Chloro-6-(chloromethyl)-2-cyclopropylpyrimidine (**18b**)*

Compound **18b** was obtained as a white solid (yield 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (s, 1H), 4.51 (s, 2H), 2.22 (s, 1H), 1.20-1.05 (m, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.5, 166.3, 162.1, 116.1, 44.9, 18.3, 11.8. MS (ESI/APCI) *m/z* 202.9 $[M+H]^+$.

*4.1.9.3. 4-Chloro-6-(chloromethyl)-2-(methylthio)pyrimidine (**18c**)*

Compound **18c** was obtained as a white solid (yield 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (s, 1H), 4.51 (s, 2H), 2.57 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.7, 166.6, 162.1, 114.4, 44.6, 14.4. MS (ESI/APCI) *m/z* 208.8 $[M+H]^+$.

*4.1.10. General procedure for the synthesis of **19a-c***

A mixture of *N*-methylquinolin-8-amine (3 mmol, 1.0 eq.), **18a**/**b**/**c** (1.2 eq.), KI (0.1 eq.) and K_2CO_3 (2.0 eq.) in CH_3CN (10 mL) was stirred at reflux overnight. The reaction solution was evaporated to remove most of CH_3CN. The residue was purified by silica gel column chromatography (dichloromethane/methanol/ammonium hydroxide = 100/1/1) to give the desired product.

*4.1.10.1. N-((6-Chloro-2-ethylpyrimidin-4-yl)methyl)-N-methylquinolin-8-amine (**19a**)*

Compound **19a** was obtained as a yellow oil (yield 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (d, *J* = 2.8 Hz, 1H), 8.11 (d, *J* = 8.0 Hz, 1H), 7.61 (s, 1H), 7.46-7.34 (m, 3H), 7.11 (d, *J* = 7.2 Hz, 1H), 4.86 (s, 2H), 3.05 (s, 3H), 2.95 (q, *J* = 7.6 Hz, 2H), 1.36 (q, *J* = 7.6 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.7, 171.9, 161.4, 148.3, 147.7, 142.3, 136.6, 129.9, 126.8, 121.1, 120.6, 117.2, 115.4, 61.7, 41.0, 32.6, 12.8. MS (ESI/APCI) *m/z* 312.8 $[M+H]^+$.

*4.1.10.2. N-((6-Chloro-2-cyclopropylpyrimidin-4-yl)methyl)-N-methylquinolin-8-amine (**19b**)*

Compound **19b** was obtained as a yellow oil (yield 33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (d, *J* = 2.4 Hz, 1H), 8.11 (d, *J* = 8.0 Hz, 1H), 7.50-7.31 (m, 4H), 7.09 (d, *J* = 7.2 Hz, 1H), 4.85 (s, 2H), 3.04 (s, 3H), 2.25-2.13 (m, 1H), 1.16-1.09 (m, 2H), 1.08-1.01 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.7, 171.2, 161.1, 148.2, 147.6, 142.2, 136.5, 129.8, 126.7, 121.0, 120.5, 116.4, 115.5, 61.4, 40.9, 18.2, 11.2. MS (ESI/APCI) *m/z* 324.8 $[M+H]^+$.

*4.1.10.3. N-((6-Chloro-2-(methylthio)pyrimidin-4-yl)methyl)-N-methylquinolin-8-amine (**19c**)*

Compound **19c** was obtained as a yellow oil (yield 25%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.11 (d, *J* = 8.4 Hz, 1H), 7.48-7.32 (m, 4H), 7.10 (d, *J* = 7.2 Hz, 1H), 4.85 (s, 2H), 3.04 (s, 3H), 2.51 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.7, 171.8, 161.2, 148.1, 147.6, 142.2, 136.5, 129.8, 126.7, 121.1, 120.5, 115.4, 114.9, 61.4, 40.9, 14.3. MS (ESI/APCI) *m/z* 330.8 $[M+H]^+$.

*4.1.11. General procedure for the synthesis of **20-22***

A mixture of **19a**/**b**/**c** (2 mmol, 1.0 eq.), TEA (2.0 eq.) and *N*-methylpiperazine (1.2 eq.) in ethanol (10 mL) was stirred at reflux overnight. The reaction solution was evaporated. The residue was purified by silica gel column chromatography (dichloromethane/methanol/ammonium hydroxide = 100/2/1) to give the desired product.

*4.1.11.1. N-((2-Ethyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-N-methylquinolin-8-amine (**20**)*

Compound **20** was obtained as a yellow oil (yield 13%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (d, *J* = 2.4 Hz, 1H), 8.10 (d, *J* = 8.0 Hz, 1H), 7.46-7.29 (m, 3H), 7.12 (d, *J* = 6.8 Hz, 1H), 6.65 (s, 1H), 4.71 (s, 2H), 3.57 (s, 4H), 3.06 (s, 3H), 2.75 (q, *J* = 7.6 Hz, 2H), 2.41-2.35 (m, 4H), 2.29 (s, 3H), 1.30 (t, *J* = 7.6 Hz, 3H).^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8, 167.0, 162.8, 148.9, 147.7, 142.6, 136.5, 129.8, 126.8, 121.0, 120.6, 116.2, 98.0, 61.8, 54.8, 46.3, 43.8, 41.5, 32.7, 12.8. HRMS (ESI): calcd for $C_{22}H_{29}N_6$ $[M+H]^+$ 377.2448, found 377.2443. Purity: 99.5%.

*4.1.11.2. N-((2-Cyclopropyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-N-methylquinolin-8-amine (**21**)*

Compound **21** was obtained as a yellow oil (yield 21%). ^{1}H NMR (400 MHz, $CDCl_3$) δ8.78 (d, *J* = 4.4 Hz, 1H), 8.06 (d, *J* = 8.0 Hz, 1H), 7.44-7.30 (m, 3H), 7.09 (d, *J* = 7.2 Hz, 1H), 6.57 (s, 1H), 4.70 (s, 2H), 3.60-3.40 (m, 4H), 3.03 (s, 3H), 2.38-2.30 (s, 4H), 2.26 (s, 3H), 2.09-1.96 (m, 1H), 1.06-0.97 (m, 2H), 0.92-0.82 (m, 2H).^{13}C NMR (150 MHz, $CDCl_3$) δ 170.2, 166.3, 162.4, 148.8, 147.5, 142.5, 136.4, 129.7, 126.7, 120.9, 120.4, 116.1, 97.8, 61.4, 54.5, 46.1, 43.6, 41.4, 17.8, 9.7. HRMS (ESI): calcd for $C_{23}H_{29}N_6$ $[M+H]^+$ 389.2448, found 389.2448. Purity: 95.6%.

*4.1.11.3. N-Methyl-N-((6-(4-methylpiperazin-1-yl)-2-(methylthio)pyrimidin-4-yl)methyl)quinolin-8-amine (**22**)*

Compound **22** was obtained as a yellow oil (yield 13%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 8.10 (d, *J* = 8.0 Hz, 1H), 7.47-7.30 (m, 3H), 7.10 (d, *J* = 6.8 Hz, 1H), 6.46 (s, 1H), 4.70 (s, 2H), 3.56 (s, 4H), 3.05 (s, 3H), 2.54 (s, 3H), 2.42-2.35 (m, 4H), 2.29 (s, 3H).^{13}C NMR (150 MHz, $CDCl_3$) δ 170.5, 167.4, 162.1, 148.8, 147.7, 142.6, 136.5, 129.8, 126.8, 121.0, 120.5, 116.3, 96.6, 61.5, 54.7, 46.2, 43.8, 41.4, 14.2. HRMS (ESI): calcd for $C_{21}H_{27}N_6S$ $[M+H]^+$ 395.2012, found 395.1998. Purity: 99.0%.

*4.1.12. General procedure for the synthesis of **24** and **28***

To a mixture of methyl 4,4-dimethoxy-3-oxobutanoate **23** (2 mmol, 1.0 eq.), corresponding amidine (for **24**: 2-methyl-2-thiopseudourea sulfate; for **28**: 2-hydroxyacetimidamide hydrochloride) (1.2 eq.) in 10 mL of H_2O was added K_2CO_3 (3 eq.). The mixture was stirred at room temperature overnight and adjusted pH to acid 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.

*4.1.12.1. 6-(Dimethoxymethyl)-2-(methylthio)pyrimidin-4-ol (**24**)*

Compound **24** was obtained as a white solid (yield 92%). ^{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). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.6, 163.1, 162.4, 108.0, 102.1, 53.7, 13.4. MS (ESI/APCI) *m/z* 216.9 $[M+H]^+$.

*4.1.12.2. 6-(Dimethoxymethyl)-2-(hydroxymethyl)pyrimidin-4-ol (**28**)*

Compound **28** was obtained as a yellow solid (yield 31%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.17 (s, 1H), 6.21 (s, 1H), 5.63 (t, *J* = 5.6 Hz, 1H), 5.02 (s, 2H), 3.27 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.3, 162.2, 161.6, 110.1, 101.7, 61.3, 53.3. MS (ESI/APCI) *m/z* 200.9 $[M+H]^+$.

*4.1.13. General procedure for the synthesis of **25** and **29***

To a solution of **24 or 28** (16 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 (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.

*4.1.13.1. 4-(Dimethoxymethyl)-6-(4-methylpiperazin-1-yl)-2-(methylthio)pyrimidine (**25**)*

Compound **25** was obtained as a yellow oil (yield 59%). ^{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* = 5.2 Hz, 4H), 2.34 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.3, 164.1, 162.0, 103.4, 95.0, 54.7, 54.1, 46.2, 43.8, 14.2. MS (ESI/APCI) *m/z* 298.8 $[M+H]^+$.

*4.1.13.2. (4-(Dimethoxymethyl)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)methanol (**29**)*

Compound **29** was obtained as a yellow oil (yield 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.65 (s, 1H), 5.15 (s, 1H), 4.61 (s, 2H), 3.90-3.61 (m, 4H), 3.40 (s, 6H), 2.51-2.45 (s, 4H), 2.34 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.6, 164.0, 162.3, 103.2, 98.0, 64.4, 54.7, 54.0, 46.2, 43.9. MS (ESI/APCI) *m/z* 282.9 $[M+H]^+$.

*4.1.14. 4-(Dimethoxymethyl)-6-(4-methylpiperazin-1-yl)-2-(methylsulfonyl)pyrimidine (**26**)*

To a solution of **25** (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 6.84 (s, 1H), 5.16 (s, 1H), 4.00-3.60 (m, 4H), 3.41 (s, 6H), 3.28 (s, 3H), 2.52-2.44 (m, 4H), 2.33 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.5, 165.1, 162.7, 102.9, 101.6, 54.5, 54.4, 46.1, 44.3, 38.9. MS (ESI/APCI) *m/z* 330.8 $[M+H]^+$.

*4.1.15. 4-(Dimethoxymethyl)-2-methoxy-6-(4-methylpiperazin-1-yl)pyrimidine (**27a**)*

To a solution of **26** (202 mg, 0.61 mmol) in 5 mL of MeOH was added MeONa (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 as a yellow oil (151 mg, yield 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ6.43 (s, 1H), 5.05 (s, 1H), 3.92 (s, 3H), 3.70 (s, 4H), 3.41 (s, 6H), 2.49-2.44 (m, 4H), 2.33 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.2, 165.6, 164.4, 103.6, 93.6, 54.7, 54.4, 54.2, 46.2, 44.1. MS (ESI/APCI) *m/z* 282.9 $[M+H]^+$.

*4.1.16. 4-(Dimethoxymethyl)-N,N-dimethyl-6-(4-methylpiperazin-1-yl)pyrimidin-2-amine (**27b**)*

To a solution of **26** (340 mg, 1 mmol) in THF (5 mL) was added dimethylamine (5 mL) and stirred at reflux overnight in a sealed tube. Then the reaction solution was cooled to room temperature, added saturated $NaHCO_3$ aqueous solution (100 mL) and extracted with dichloromethane (100 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by silica gel column chromatography (dichloromethane/methanol/ammonium hydroxide = 100/1/1 to 100/3/1) to give the desired product (260 mg, 88%) as a yellow oil. ^{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). ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.8, 163.3, 162.4, 104.3, 88.4, 54.8, 54.0, 46.2, 43.8, 37.0. MS (ESI/APCI) *m/z* 295.9 $[M+H]^+$.

*4.1.17. General procedure for the synthesis of **30a-c***

A mixture of **27a**/**27b**/**29** (1.3 mmol, 1.0 eq.) in 5 mL of 20% H_2SO_4 was stirred at reflux overnight. The reaction mixture was adjusted 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.

*4.1.17.1. 2-Methoxy-6-(4-methylpiperazin-1-yl)pyrimidine-4-carbaldehyde (**30a**)*

Compound **30a** was obtained as a yellow oil (yield 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (s, 1H), 6.71 (s, 1H), 3.95 (s, 3H), 3.71 (s, 4H), 2.45 (s, 4H), 2.31 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 193.7, 166.4, 164.3, 159.5, 94.2, 54.7, 54.6, 46.1, 44.2. MS (ESI/APCI) *m/z* 268.9 $[M+MeOH+H]^+$.

*4.1.17.2. 2-(Dimethylamino)-6-(4-methylpiperazin-1-yl)pyrimidine-4-carbaldehyde (**30b**)*

Compound **30b** 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), 2.51-2.41 (m, 4H), 2.33 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 195.3, 163.3, 163.0, 159.1, 89.1, 54.8, 46.2, 43.9, 37.0. MS (ESI/APCI) *m/z* 250.0 $[M+H]^+$.

*4.1.17.3. 2-(Hydroxymethyl)-6-(4-methylpiperazin-1-yl)pyrimidine-4-carbaldehyde (**30c**)*

Compound **30c** was obtained as a yellow oil (yield 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.90 (s, 1H), 6.95 (s, 1H), 4.68 (s, 2H), 3.88-3.59 (m, 5H), 2.59-2.45 (m, 4H), 2.35 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 193.6, 169.1, 162.4, 157.2, 98.3, 64.4, 54.6, 46.2, 44.2. MS (ESI/APCI) *m/z* 269.0

$[M+MeOH+H]^+$.

*4.1.18. General procedure for the synthesis of **31a-c***

A mixture of **30a**/**b**/**c** (0.66 mmol, 1.0 eq.), quinolin-8-amine (1.5 eq.), and AcOH (1 drop) in methanol (5 mL) was stirred for 30 min. $NaBH_3CN$ (2.0 eq.) was then added to the reaction solution. The resulting suspension was stirred at room temperature for 6h. The reaction solution was evaporated. The residue was purified by silica gel column chromatography (dichloromethane/methanol/ammonium hydroxide = 200/2/1) to give the desired product.

*4.1.18.1. N-((2-Methoxy-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)quinolin-8-amine (**31a**)*

Compound **31a** was obtained as a yellow oil (yield 65 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (dd, *J* = 8.4 Hz, 1.6 Hz, 1H), 8.06 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.40 (dd, *J* = 8.4, 4.0 Hz, 1H), 7.34-7.29 (m, 1H), 7.07 (dd, *J* = 8.4, 1.2 Hz, 1H), 6.84-6.75 (m, 1H), 6.58 (dd, *J* = 7.6, 1.2 Hz, 1H), 6.31 (s, 1H), 4.43 (d, *J* = 5.6 Hz, 2H), 3.95 (s, 3H), 3.67-3.46 (m, 4H), 2.36 (t, *J* = 5.2 Hz, 4H), 2.26 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.9, 165.5, 164.4, 147.1, 144.5, 138.4, 136.1, 128.6, 127.8, 121.5, 114.7, 105.7, 93.1, 54.6, 54.3, 49.3, 46.1, 44.0. MS (ESI/APCI) *m/z* 364.9 $[M+H]^+$.

*4.1.18.2. N-((2-(Dimethylamino)-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)quinolin-8-amine (**31b**)*

Compound **31b** was obtained as a yellow oil (yield 42 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (d, *J* = 3.2 Hz, 1H), 8.06 (d, *J* = 8.4 Hz, 1H), 7.41-7.31 (m, 2H), 7.06 (d, *J* = 8.4 Hz, 1H), 6.96-6.89 (m, 1H), 6.63 (d, *J* = 7.6 Hz, 1H), 5.98 (s, 1H), 4.36 (d, *J* = 5.2 Hz, 2H), 3.56 (s, 4H), 3.19 (s, 6H), 2.40 (s, 4H), 2.29 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.9, 163.4, 162.3, 147.1, 145.0, 138.5, 136.1, 128.7, 127.9, 121.5, 114.2, 105.7, 88.4, 54.9, 49.4, 46.3, 43.9, 37.0. MS (ESI/APCI) *m/z* 377.9 $[M+H]^+$.

*4.1.18.3. (4-(4-Methylpiperazin-1-yl)-6-((quinolin-8-ylamino)methyl)pyrimidin-2-yl)methanol (**31c**)*

Compound **31c** was obtained as a yellow oil (yield 90 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (d, *J* = 3.2 Hz, 1H), 8.09 (d, *J* = 8.0 Hz, 1H), 7.44-7.37 (m, 1H), 7.36-7.30 (m, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 6.83-6.75 (m, 1H), 6.55 (d, *J* = 7.6 Hz, 1H), 6.52 (s, 1H), 4.62 (s, 2H), 4.50 (d, *J* = 6.0 Hz, 2H), 3.59 (s, 4H), 2.44-2.35 (m, 4H), 2.28 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.4, 166.7, 162.4, 147.3, 144.4, 138.4, 136.2, 128.7, 127.8, 121.6, 115.0, 105.8, 97.3, 64.3, 54.6, 49.3, 46.2, 43.9. MS (ESI/APCI) *m/z* 364.9 $[M+H]^+$.

*4.1.19. General procedure for the synthesis of **32-34***

A mixture of **31a**/**b**/**c** (0.28 mmol, 1.0 eq.) and formaldehyde (30 wt percent in water, 5.0 eq.) in methanol (5 mL) was stirred for 2h. Then $NaBH_3CN$ (2.0 eq.) was added. The resulting suspension was stirred at room temperature overnight. The reaction solution was evaporated. The residue was purified by silica gel column chromatography (dichloromethane/methanol/ammonium hydroxide = 200/1/1) to give the desired product.

*4.1.19.1. N-((2-Methoxy-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-N-methylquinolin-8-amine (**32**)*

Compound **32** was obtained as a yellow oil (yield 13%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (d, *J* = 2.4 Hz, 1H), 8.06 (d, *J* = 8.0 Hz, 1H), 7.41-7.28 (m, 3H), 7.09 (d, *J* =7.2 Hz, 1H), 6.37 (s, 1H), 4.67 (s, 2H), 3.83 (s, 3H), 3.52 (s, 4H), 3.04 (s, 3H), 2.39-2.31 (m, 4H), 2.25 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.1, 165.1, 164.1, 148.7, 147.5, 142.4, 136.4, 129.7, 126.6, 120.8, 120.4, 116.2, 94.8, 61.4, 54.5, 54.0, 46.1, 43.9, 41.4. HRMS (ESI): calcd for $C_{21}H_{27}N_6O$ $[M+H]^+$ 379.2241, found 379.2252. Purity: 96.5%.

*4.1.19.2. N-((2-(Dimethylamino)-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-N-methylquinolin-8-amine (**33**)*

Compound **33** was obtained as a yellow oil (yield 27%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 7.45-7.28 (m, 3H), 7.12 (d, *J* = 7.2 Hz, 1H), 6.08 (s, 1H), 4.69 (s, 2H), 3.58 (s, 4H), 3.15-2.95 (m, 9H), 2.45 (s, 4H), 2.33 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.7, 162.8, 160.7, 148.9, 147.5, 142.4, 136.7,129.8, 126.8, 120.9, 120.4, 116.6, 90.8, 61.0, 54.5, 45.9, 43.7, 41.2, 37.1. HRMS (ESI): calcd for $C_{22}H_{30}N_7$ $[M+H]^+$ 392.2557, found 392.2557. Purity: 98.7%.

*4.1.19.3. (4-((Methyl(quinolin-8-yl)amino)methyl)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)methanol (**34**)*

Compound **34** was obtained as a yellow oil (yield 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (d, *J* = 2.4 Hz, 1H), 8.06 (d, *J* = 8.0 Hz, 1H), 7.43-7.28 (m, 3H), 7.08 (d, *J* = 7.2 Hz, 1H), 6.67 (s, 1H), 4.72 (s, 2H), 4.53 (s, 2H), 3.82 (br s, 1H), 3.55 (m, 4H), 3.01 (s, 3H), 2.40-2.30 (m, 4H), 2.25 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.9, 166.7, 162.1, 148.6, 147.5, 142.3, 136.4, 129.7, 126.6, 120.9, 120.5, 116.0, 98.9, 64.1, 61.4, 54.4, 46.0, 43.7, 41.1. HRMS (ESI): calcd for $C_{21}H_{27}N_6O$ $[M+H]^+$ 379.2241, found 379.2249. Purity: 97.5%.

*4.1.20. N-((6-Chloro-2-methylpyrimidin-4-yl)methyl)quinolin-8-amine (**35**)*

A mixture of **9** (273 mg, 1.5 mmol), quinolin-8-amine (200 mg, 1.4 mmol), KI (23 mg, 0.14 mmol) and K_2CO_3 (386 mg, 2.8 mmol) in CH_3CN (5 mL) was stirred at reflux overnight. The reaction solution was evaporated to remove most of CH_3CN, diluted with saturated $NaHCO_3$ (50 mL) and extracted with dichloromethane (20 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/ammonium hydroxide = 100/2/1) to give the desired product (230 mg, 58%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84-8.74 (m, 1H), 8.11 (d, *J* = 8.4 Hz, 1H), 7.43 (dd, *J* = 8.4, 4.0 Hz, 1H), 7.37-7.30 (m, 1H), 7.28 (s, 1H), 7.14 (d, *J* = 8.4 Hz, 1H), 6.96-6.83 (m, 1H), 6.47 (d, *J* = 7.6 Hz, 1H), 4.65(d, *J* = 6.4 Hz, 1H), 2.76 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8, 169.1, 162.0, 147.5, 143.7, 138.3,136.3, 128.8, 127.7, 121.8, 115.5, 115.4, 105.4, 48.8, 25.9. MS (ESI/APCI) *m/z* 284.8 $[M+H]^+$.

*4.1.21. N-((2-Methyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)quinolin-8-amine (**36**)*

A mixture of **35** (100 mg, 0.35 mmol), TEA (354 mg, 3.5 mmol) and *N*-methylpiperazine (42 mg, 0.42 mmol) in ethanol (4 mL) was stirred at reflux overnight. The reaction mixture was concentrated and added saturated $NaHCO_3$ aqueous solution (20 mL), then 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/ammonium hydroxide = 100/1/1) to give the desired product (75 mg, 56 %) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (d, *J* = 2.8 Hz, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 7.41 (dd, *J* = 8.0, 4.0 Hz, 1H), 7.36-7.31 (m, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 6.80-6.74 (m, 1H), 6.57 (d, *J* = 7.6 Hz, 1H), 6.48 (s, 1H), 4.49 (d, *J* = 6.0 Hz, 2H), 3.58 (s, 4H), 2.55 (s, 3H), 2.42-2.35 (m, 4H), 2.28 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.3, 166.7, 162.9, 147.2, 144.6, 138.4, 136.2, 128.7, 127.9, 121.6, 114.8, 105.8, 96.0, 54.7, 49.5, 46.2, 43.7, 26.2. MS (ESI/APCI) *m/z* 348.9 $[M+H]^+$.

*4.1.22. N-Ethyl-N-((2-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)quinolin-8-amine (**37**)*

A mixture of **36** (80 mg, 0.23 mmol) and acetaldehyde (37 wt percent in water, 137 mg, 1.2 mmol) in methanol (10 mL) was added $NaBH_3CN$ (36 mg, 0.58 mmol). The resulting suspension was stirred at room temperature overnight. Water (10 mL) was added to quench the reaction. The water phase was 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/ammonium hydroxide = 100/1/1) to give the desired product (20 mg, 22%)

as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (d, *J* = 2.4 Hz, 1H), 8.10 (d, *J* = 8.0 Hz, 1H), 7.42-7.29 (m, 3H), 7.17-7.10 (m, 1H), 6.58 (s, 1H), 4.61 (s, 2H), 3.65 (q, *J* = 7.2 Hz, 2H), 3.45 (s, 4H), 2.49 (s, 3H), 2.34-2.28 (m, 4H), 2.25 (s, 3H), 1.12 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.1, 166.6, 162.6, 147.9, 146.8, 143.4, 136.6,130.0, 126.6, 121.0, 120.9, 118.7, 97.9, 58.3, 54.7, 48.6, 46.2, 43.7, 26.2, 11.9. HRMS (ESI): calcd for $C_{22}H_{29}N_6$ $[M+H]^+$ 377.2448, found 377.2451. Purity: 95.9%.

*4.1.23. N-Cyclopropylquinolin-8-amine (**39**)*

The mixture of **38** (104 mg, 0.5 mmol), cyclopropanamine (300 mg, 2.5 mmol), Cs_2CO_3 (326 mg, 1 mmol), $Pd_2(dba)_3$ (22 mg, 0.025 mol) and BINAP (31 mg, 0.05 mmol) in toluene (10 mL) was stirred at 100°C overnight under N_2 atmosphere. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 50/1) to give the desired product (50 mg, 54%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (dd, *J* = 4.0, 1.6 Hz,1H), 8.07 (dd, *J* = 8.4, 1.6 Hz,1H), 7.48-7.40 (m, 1H), 7.36 (dd, *J* = 8.0, 4.0 Hz,1H), 7.11 (s, 1H), 7.09 (s, 1H), 6.42 (s, 1H), 2.62-2.54 (m, 1H), 0.87-0.80 (m, 2H), 0.70-0.64 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 147.0, 145.4, 136.5, 136.2, 128.7, 127.9, 121.4, 114.6, 106.5, 24.8, 7.3. MS (ESI/APCI) *m/z* 184.9 $[M+H]^+$.

*4.1.24. N-((6-Chloro-2-methylpyrimidin-4-yl)methyl)-N-cyclopropylquinolin-8-amine (**40**)*

A mixture of **39** (50 mg, 0.27 mmol), **9** (62 mg, 0.81 mmol), KI (5 mg, 0.03 mmol) and K_2CO_3 (75 mg, 0.54 mmol) in CH_3CN (10 mL) was stirred at reflux overnight. The reaction solution was evaporated to remove most of CH_3CN, diluted with saturated $NaHCO_3$ (50 mL) and extracted with dichloromethane (20 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 = 40/1) to give the desired product (70 mg, 79%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (d, *J* = 2.4 Hz, 1H), 8.10 (d, *J* = 8.0 Hz, 1H), 7.54-7.45 (m, 2H), 7.44-7.38 (m, 1H), 7.37-7.31 (m, 2H), 5.19 (s, 2H), 2.83-2.75 (m, 1H), 2.67 (s, 3H), 0.85-0.78 (m, 2H), 0.68-0.55 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.8, 168.3, 160.6, 147.7, 147.4, 141.8, 136.8, 129.7, 126.8, 121.0, 120.7, 117.5, 117.4, 60.8, 34.6, 25.9, 9.3. MS (ESI/APCI) *m/z* 324.8 $[M+H]^+$.

*4.1.25. N-Cyclopropyl-N-((2-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)quinolin-8-amine (**41**)*

A mixture of **40** (70 mg, 0.25 mmol), TEA (250 mg, 2.5 mmol) and *N*-methylpiperazine (75 mg, 0.75 mmol) in ethanol (4 mL) was stirred at reflux overnight. The reaction mixture was concentrated and added saturated $NaHCO_3$ aqueous solution (20 mL), then 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/ammonium hydroxide = 100/1/1) to give the desired product (40 mg, 41%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (d, *J* = 2.4 Hz, 1H), 8.08 (d, *J* = 8.0 Hz, 1H), 7.50-7.40 (m, 2H), 7.39-7.29 (m, 2H), 6.21 (s, 1H), 5.14 (s, 2H), 3.50-3.35 (m, 4H), 2.84-3.75 (m, 1H), 2.45 (s, 3H), 2.38-2.31 (m, 4H), 2.27 (s, 3H), 0.84-0.74 (m, 2H), 0.70-0.61 (m, 2H).^{13}C NMR (150 MHz, $CDCl_3$) δ 167.8, 166.7, 162.3, 148.3, 147.4, 142.1, 136.5,129.7, 126.5, 120.8, 120.3, 117.9, 98.2, 60.3, 54.7, 46.2, 43.7, 34.1, 26.2, 9.5. HRMS (ESI): calcd for $C_{23}H_{29}N_6$ $[M+H]^+$ 389.2448, found 389.2448. Purity: 99.4%.

*4.1.26. 6-(Dimethoxymethyl)-2,5-dimethylpyrimidin-4-ol (**43**)*

To a mixture of methyl 4,4-dimethoxy-2-methyl-3-oxobutanoate **42** (670 mg, 3.5 mmol), acetamidine hydrochloride (994 mg, 10.6 mmol) in 10 mL of H_2O was added K_2CO_3 (966 mg, 7 mmol). The mixture was stirred at room temperature overnight and adjusted pH to acid 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 = 100/1) to give the desired product (290 mg, 42%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.06 (s, 1H), 5.27 (s, 1H), 3.43 (s, 6H), 2.48 (s, 3H), 2.13 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.2, 157.8, 155.8, 119.8, 103.1, 54.8, 21.7, 9.7. MS (ESI/APCI) *m/z* 198.9 $[M+H]^+$.

*4.1.27. 4-(Dimethoxymethyl)-2,5-dimethyl-6-(4-methylpiperazin-1-yl)pyrimidine (**44**)*

To a solution of **43** (290 mg, 1.5 mmol), Et_3N (1.5 g, 14.6 mmol) and *N*-methyl piperazine (293 mg, 2.9 mmol) in 10 mL of MeCN was added PyBOP (838 mg, 1.6 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 (240 mg, 59%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.27 (s, 1H), 3.43 (s, 6H), 3.38-3.32 (m, 4H), 2.57-2.47 (m, 7H), 2.34 (s, 3H), 2.22 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.1, 163.9, 161.7, 113.8, 105.4, 55.1, 54.9, 48.5, 46.3, 25.9, 13.6. MS (ESI/APCI) *m/z* 280.9 $[M+H]^+$.

*4.1.28. 2,5-Dimethyl-6-(4-methylpiperazin-1-yl)pyrimidine-4-carbaldehyde (**45**)*

A mixture of **44** (100 mg, 0.36 mmol) in 5 mL of 20% H_2SO_4 was stirred at reflux overnight. The reaction mixture was adjusted 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 (50 mg, 60%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1H), 3.49-3.40 (m, 4H), 2.62 (s, 3H), 2.56-2.52 (m, 4H), 2.39 (s, 3H), 2.35 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 195.8, 167.7, 164.9, 155.1, 116.2, 55.0, 48.5, 46.2, 25.6, 14.6. MS (ESI/APCI) *m/z* 266.9 $[M+MeOH+H]^+$.

*4.1.29. N-((2,5-Dimethyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)quinolin-8-amine (**46**)*

A mixture of **45** (150 mg, 0.63 mmol), quinolin-8-amine (144 mg, 1.0 mmol), and AcOH (1 drop) in methanol (5 mL) was stirred for 30 min. $NaBH_3CN$ (144 mg, 2.0 mmol) was then added to the reaction solution. The resulting suspension was stirred at room temperature for 6h. The reaction solution was evaporated. The residue was purified by silica gel column chromatography (dichloromethane/methanol/ammonium hydroxide = 200/2/1) to give the desired product (52 mg, 22%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (d, *J* = 2.0 Hz, 1H), 8.07 (d, *J* = 8.0 Hz, 1H), 7.44-7.36 (m, 2H), 7.09 (d, *J* =8.0 Hz, 1H), 6.75 (d, *J* =7.2 Hz, 1H), 4.45 (s, 2H), 3.63 (s, 4H), 2.93 (s, 4H), 2.65 (s, 3H), 2.61 (s, 3H), 2.23 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.1, 164.1, 163.1, 147.3, 144.7, 138.7, 136.0, 128.8, 127.8, 121.5, 114.3, 112.8, 105.1, 55.2, 48.6, 46.6, 46.3, 26.0, 13.8. MS (ESI/APCI) *m/z* 362.9 $[M+H]^+$.

*4.1.30. N-((2,5-Dimethyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-N-methylquinolin-8-amine (**47**)*

A mixture of **46** (36 mg, 0.10 mmol) and formaldehyde (30 wt percent in water, 50 mg, 0.5 mmol) in methanol (5 mL) was stirred for 0.5 h. Then $NaBH_3CN$ (62 mg, 1 mmol) was added. The resulting suspension was stirred at room temperature overnight. The reaction solution was evaporated. The residue was purified by silica gel column chromatography (dichloromethane/methanol/ammonium hydroxide = 200/1/1) to give the desired product (16 mg, 42%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (d, *J* = 2.4 Hz, 1H), 8.11 (d, *J* = 8.0 Hz, 1H), 7.45-7.32 (m, 3H), 7.11 (d, *J* = 4.4 Hz, 1H), 4.85 (s, 2H), 3.24 (s, 4H), 3.05 (s, 3H), 2.53 (s, 3H), 2.47 (s, 4H), 2.31 (s, 3H), 1.86 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.5, 164.3, 163.4, 149.4, 147.7, 143.0, 136.6, 129.7, 126.7, 120.9, 120.8, 117.5, 114.6, 59.5, 55.1, 48.5, 46.3, 41.6, 25.8, 14.2. HRMS (ESI): calcd for $C_{22}H_{29}N_6$ $[M+H]^+$ 377.2448, found 377.2445. Purity: 99.6%.

*4.1.31. Ethyl 2,4-difluoro-3-oxobutanoate (**49**)*

To a suspension of NaH (60% dispersion in mineral oil, 936 mg, 24 mmol) in 50 mL of ether was added **48** (5.0 g, 47 mmol) at room temperature. The mixture was stirred at reflux 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. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 10/1) to give the desired product (2 g, 25%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.45 (d, *J* = 48.0 Hz, 0.86H), 5.19 (d, *J* = 46.8 Hz, 1.68H), 4.89 (d, *J* = 47.2 Hz, 0.42H), 4.31 (q, *J* = 7.2 Hz, 2H), 1.31 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 195.4 (dd, *J* = 22.4, 17.4 Hz), 163.3 (d, *J* = 23.3 Hz), 89.7 (d, *J* = 194.7 Hz), 83.3 (dd, *J* = 183.3, 3.3 Hz), 63.3, 13.9. MS (ESI/APCI) *m/z* 164.9 $[M-H]^-$.

*4.1.32. 5-Fluoro-6-(fluoromethyl)-2-methylpyrimidin-4-ol (**50**)*

A mixture of **49** (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 reflux overnight. Then 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 13.22 (s, 1H), 5.35 (d, *J* = 46.8 Hz, 2H), 2.52 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.4 (d, *J* = 24.2 Hz), 154.6 (d, *J* = 6.9 Hz), 146.4 (dd, *J* = 256.8, 3.0 Hz), 144.5 (dd, *J* = 15.8, 10.4 Hz), 78.0 (d, *J* = 171.2 Hz), 21.5. MS (ESI/APCI) *m/z* 158.9 $[M-H]^-$.

*4.1.33. 5-Fluoro-4-(fluoromethyl)-2-methyl-6-(4-methylpiperazin-1-yl)pyrimidine (**51**)*

To a solution of **50** (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 5.30 (dd, *J* = 47.2 Hz, 2.4 Hz, 2H), 3.79-3.73 (m, 4H), 2.46-2.39 (m, 4H), 2.26 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.3 (d, *J* = 8.6 Hz), 151.4 (d, *J* = 4.1 Hz), 147.3 (t, *J* = 15.3 Hz), 143.7 (dd, *J* = 258.8, 3.8 Hz), 79.1 (d, *J* = 168.9 Hz), 54.9, 46.0 (d, *J* = 12.8 Hz), 45.9, 25.3. MS (ESI/APCI) *m/z* 242.9 $[M+H]^+$.

*4.1.34. N-((5-Fluoro-2-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-N-methylquinolin-8-amine (**52**)*

A mixture of **51** (48mg, 0.2 mmol), *N*-methylquinolin-8-amine (63 mg, 0.4 mmol), KI (6 mg, 0.036 mmol) and 2-ethyl-2-(hydroxymethyl)propane-1,3-diol (295 mg, 2.2 mmol) in water (1 mL) was stirred at reflux overnight. The reaction mixture was diluted with saturated $NaHCO_3$ (10 mL) and extracted with dichloromethane (20 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 = 50/1) to give the product (20 mg, 13%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (d, *J* = 4.0 Hz, 1H), 8.08 (d, *J* = 8.0 Hz, 1H), 7.44-7.29 (m, 3H), 7.01 (s, 1H), 4.95 (s, 2H), 3.67-3.48 (m, 4H), 3.06 (s, 3H), 2.47-2.32 (m,7H), 2.26 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.3 (d, *J* = 8.6 Hz), 151.4 (d, *J* = 5.1 Hz), 150.7 (d, *J* = 15.6 Hz), 148.8, 147.7, 143.9 (d, *J* = 255.2 Hz), 142.9, 136.4, 129.5, 126.4, 120.9, 120.8, 117.3, 55.0, 46.2, 46.0, 45.9, 40.9, 25.5 (d, *J* = 1.8 Hz). HRMS (ESI): calcd for $C_{21}H_{26}FN_6$ $[M+H]^+$ 381.2197, found 381.2182. Purity: 95.3%.

*4.1.35. 5-Chloro-6-(chloromethyl)-2-methylpyrimidin-4-ol (**54**)*

To a solution of ethyl **53** (500mg, 2.5 mmol) and acetamidine hydrochloride (262mg, 2.8 mmol) in water (20 mL) was added K_2CO_3 (524 mg, 3.8 mmol). The reaction was stirred at room temperature overnight. The reaction was quenched with 1M HCl aqueous solution to adjust pH to 5 and then extracted

with ethyl acetate (15 mL x 4). The combined organic layer was dried over Na_2SO_4, concentrated and was purified by silica gel column chromatography (dichloromethane/methanol = 100/1) to give the desired product (140 mg, 29%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.88 (s, 1H), 4.56 (s, 2H), 2.55 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.4, 159.1, 157.1, 119.5, 43.0, 21.6. MS (ESI/APCI) *m/z* 192.8 $[M+H]^+$.

*4.1.36. 4,5-Dichloro-6-(chloromethyl)-2-methylpyrimidine (**55**)*

The solution of **54** (110 mg, 0.57 mmol) in $POCl_3$ (2 mL) was stirred at reflux for 1 h. The reaction mixture was concentrated, added water (20 mL) and extracted with ethyl acetate (2 mL x 2). The combined organic layer was concentrated and the residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 10/1) to give the desired product (53 mg, 42%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.68 (s, 2H), 2.71 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.3, 162.8, 159.5, 126.2, 43.3, 25.3. MS (ESI/APCI) *m/z* 210.8 $[M+H]^+$.

*4.1.37. N-((5,6-Dichloro-2-methylpyrimidin-4-yl)methyl)-N-methylquinolin-8-amine (**56**)*

A mixture of **55** (53 mg, 0.25 mmol), *N*-methylquinolin-8-amine (40 mg, 0.25 mmol), KI (5 mg, 0.03 mmol) and K_2CO_3 (70 mg, 0.50 mmol) in CH_3CN (10 mL) was stirred at reflux for 2h. The reaction mixture was filtered and the filtrate was evaporated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 100/1) to give the desired product (60 mg, 72%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (d, *J* = 2.4 Hz, 1H), 8.09 (d, *J* = 7.6 Hz, 1H), 7.46-7.39 (m, 1H), 7.39-7.32 (m, 2H), 7.16 (d, *J* = 7.2 Hz, 1H), 5.24 (s, 2H), 3.25 (s, 3H), 2.60 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.1, 165.3, 158.0, 148.1, 147.4, 142.1, 136.7, 129.7, 126.7, 125.0, 120.9, 120.4, 116.7, 58.4, 41.6, 25.4. MS (ESI/APCI) *m/z* 332.7 $[M+H]^+$.

*4.1.38. N-((5-Chloro-2-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)methyl)-N-methylquinolin-8-amine (**57**)*

A mixture of **56** (57 mg, 0.17 mmol), TEA (172 mg, 1.7 mmol) and *N*-methylpiperazine (86 mg, 0.86 mmol) in ethanol (5 mL) was stirred at reflux overnight. The reaction mixture was concentrated. The residue was purified by silica gel column chromatography (dichloromethane/methanol/ammonium hydroxide = 100/2/1) to give the desired product (40 mg, 40%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84 (d, *J* = 2.4 Hz, 1H), 8.09 (d, *J* = 8.0 Hz, 1H), 7.42-7.33 (m, 3H), 7.11 (d, *J* = 6.8 Hz, 1H), 5.10 (s, 2H), 3.53 (s, 4H), 3.16 (s, 3H), 2.52-2.46 (m, 4H), 2.44 (s, 3H), 2.32 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.8, 163.7, 161.4, 149.1, 147.5, 142.8, 136.5, 129.6, 126.5, 120.8, 120.4, 117.3, 114.2, 58.0, 54.9, 47.8, 46.1, 41.4, 25.5. HRMS (ESI): calcd for $C_{21}H_{26}ClN_6$ $[M+H]^+$ 397.1902, found 397.1911. Purity: 96.2%.

*4.1.39. 4-Hydroxy-2,6-dimethylpyrimidine-5-carbonitrile (**59**)*

A mixture of **58** (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 to pH 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 $[M+H]^+$.

*4.1.40. tert-Butyl 4-(5-cyano-2,6-dimethylpyrimidin-4-yl)piperazine-1-carboxylate (**60**)*

A mixture of **59** (2.5 g, 16 mmol), *N*-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 4.01-3.87 (m, 4H), 3.62-3.48 (m, 4H), 2.57 (s, 3H), 2.51 (s, 3H), 1.48 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.8, 168.8, 161.9, 154.7, 117.6, 87.2, 80.6, 46.3, 43.9, 28.5, 26.5, 23.9. MS (ESI/APCI) *m/z* 317.9 $[M+H]^+$.

*4.1.41. tert-Butyl 4-(6-(chloromethyl)-5-cyano-2-methylpyrimidin-4-yl)piperazine-1-carboxylate (**61**)*

To a solution of **60** (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 4.57 (s, 2H), 3.98 (d, *J* = 5.2 Hz, 4H), 3.57 (d, *J* = 5.2 Hz, 4H), 2.55 (s, 3H), 1.48 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.0, 169.8, 161.8, 154.6, 116.2, 86.6, 80.7, 46.4, 44.2, 42.9, 28.5, 26.5. MS (ESI/APCI) *m/z* 351.8 $[M+H]^+$.

*4.1.42. tert-Butyl 4-(5-cyano-2-methyl-6-((methyl(quinolin-8-yl)amino)methyl)pyrimidin-4-yl)piperazine-1-carboxylate (**62**)*

A mixture of **61** (85mg, 0.24 mmol), *N*-methylquinolin-8-amine (38 mg, 0.24 mmol), KI (5 mg, 0.03 mmol) and K_2CO_3 (70 mg, 0.5 mmol) in 10 mL of MeCN was stirred at reflux for 2h. The reaction solution was cold to room temperature and filtered. The filtrate was concentrated. The resulting residue was purified by silica gel column chromatography (dichloromethane/methanol = 100/1) to give the desired product (20 mg, 24%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 8.10 (d, *J* = 7.2 Hz, 1H), 7.51-7.34 (m, 3H), 7.09 (s, 1H), 5.12 (s, 2H), 3.82 (s, 4H), 3.67-3.40 (m, 4H), 3.18 (s, 3H), 2.47 (s, 3H), 1.47 (s, 9H). MS (ESI/APCI) *m/z* 473.9 $[M+H]^+$.

*4.1.43. 2-Methyl-4-((methyl(quinolin-8-yl)amino)methyl)-6-(4-methylpiperazin-1-yl)pyrimidine-5-carbonitrile (**63**)*

To a solution of **62** (300 mg, 0.63 mmol) in 2 mL of dichloromethane was added CF_3CO_2H (1 mL). The mixture was stirred at room temperature for 2 h and then evaporated to give the residue as a red oil. To a solution of this crude product in 1 mL of methanol was added formaldehyde (30 wt percent in water, 1 mL) and $NaBH_3CN$ (65 mg, 1.0 mmol). 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 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 1/10) to give the desired product (80 mg, 33%) as a slight yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (d, *J* = 2.8 Hz, 1H), 8.07 (d, *J* = 8.0 Hz, 1H), 7.42-7.30 (m, 3H), 7.08 (d, *J* = 5.6 Hz, 1H), 5.09 (s, 2H), 3.90-3.82 (m, 4H), 3.16 (s, 3H), 2.48-2.38 (m, 7H), 2.30 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.0, 168.5, 162.3, 148.3, 147.6, 142.6, 136.4, 129.7, 126.4, 120.9, 120.7, 116.9, 116.8, 86.3, 59.6, 54.8, 46.5, 46.0, 41.6, 26.6. HRMS (ESI): calcd for $C_{22}H_{26}N_7$ $[M+H]^+$ 388.2244, found 388.2248. Purity: 98.9%.

*4.1.44. 4,6-Dichloro-2-methylpyrimidine-5-carbaldehyde (**65**)*

To $POCl_3$ (60.6 g, 397 mmol) was added dropwise DMF (9.8 g, 135 mmol) at 0°C. The resulting suspension was stirred at the same temperature for 1h. Then 2-methylpyrimidine-4,6-diol **64** (10 g, 79 mmol) was added in portions and stirred at room temperature for 1h, followed by stirring at reflux overnight. The reaction solution was concentrated and diluted with cold ethyl acetate (100mL). This solution was added dropwise into ice-water and filtered. The filtrate was extracted with ethyl acetate (200 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated to give the desired product (9 g, 59%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.41 (s, 1H), 2.75 (s, 3H). ^{13}C NMR (150

MHz, $CDCl_3$) δ 185.7, 171.6, 162.6, 121.9, 26.1. MS (ESI/APCI) *m/z* 222.8 $[M+MeOH+H]^+$.

*4.1.45. (4,6-Dichloro-2-methylpyrimidin-5-yl)methanol (**66**)*

To the solution of **65** (9 g, 47.1 mmol) in THF (50 mL) and water (10 mL) was added $NaBH_4$ (3.6 g, 94.2 mmol) in portions at 0°C. The resulting suspension was stirred at the same temperature for 30min. Water (50 mL) was added, and extracted with ethyl acetate (50 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 10/1) to give the desired product (4.2 g, 46%) as a yellow solid. ^1H NMR (400 MHz, $CDCl_3$) δ 4.89 (s, 2H), 2.67 (s, 3H), 2.51 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.4, 162.2, 127.1, 59.0, 25.4. MS (ESI/APCI) *m/z* 192.8 $[M+H]^+$.

*4.1.46. 5-((tert-Butyldimethylsilyloxy)methyl)-4,6-dichloro-2-methylpyrimidine (**67**)*

To the solution of **66** (2.1 g, 10.9 mmol) and imidazole (814 mg, 12.0 mmol) in dichloromethane (20mL) was added TBSCl (1.78 g, 12.0 mmol) in portions. The resulting suspension was stirred at room temperature overnight. Water (20 mL) was added, and extracted with dichloromethane (20 mL). The combined organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 100/1) to give the desired product (2.6 g, 78 %) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 4.85 (s, 2H), 2.68 (s, 3H), 0.91 (s, 9 H), 0.14 (s, 6H).^{13}C NMR (150 MHz, $CDCl_3$) δ 168.0, 162.4, 127.4, 59.7, 25.9, 25.5, 18.6, -5.3. MS (ESI/APCI) *m/z* 192.9 $[M-TBS+2H]^+$.

*4.1.47. 5-(((tert-Butyldimethylsilyl)oxy)methyl)-4-chloro-2-methyl-6-vinylpyrimidine (**68**)*

To the solution of **67** (2.1 g, 6.84 mmol), potassium vinyltrifluoroborate (917 mg, 6.84 mmol), and Cs_2CO_3 (4.46 g, 13.68 mmol) in 1,4-dioxane (60 mL) and water (12 mL) was added $Pd(PPh_3)_4$ (790 mg, 0.68 mmol) under N_2 atmosphere. The resulting suspension was stirred at reflux overnight. The reaction mixture was filtered and the filtrate was concentrated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 100/1) to give the desired product (1.3 g, 64 %) as a yellow oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.11 (dd, *J* = 16.8, 10.8 Hz, 1H), 6.67 (d, *J* = 16.8 Hz, 1H), 5.73 (d, *J* = 10.8 Hz, 1H), 4.85 (s, 2H), 2.68 (s, 3H), 0.89 (s, 9 H), 0.11 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.2, 163.5, 161.4, 131.7, 125.3, 124.9, 58.5, 25.9, 25.8, 18.4, -5.1. MS (ESI/APCI) *m/z* 184.9 $[M-TBS+2H]^+$.

*4.1.48. 5-(((tert-Butyldimethylsilyl)oxy)methyl)-6-chloro-2-methylpyrimidine-4-carbaldehyde (**69**)*

The intermediate **68** (1.3 g, 4.35 mmol) was dissolved in methanol (120 mL) and dichloromethane (30 mL). Then ozone was introduced into this reaction solution at -65°C and stirred at this temperature for 4h before dimethyl sulfide (3 mL) was added to quench the reaction. The resulting solution was evaporated to remove the solvent. The residue was added water (20 mL) and extracted with dichloromethane (50 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 20/1) to give the desired product (600 mg, 46 %) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 10.16 (s, 1H), 5.09 (s, 2H), 2.79 (s, 3H), 0.89 (s, 9 H), 0.13 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 191.8, 168.6, 163.4, 157.6, 128.5, 57.0, 25.9, 25.6, 18.5, -5.3. MS (ESI/APCI) *m/z* 300.9 $[M+H]^+$.

*4.1.49. N-((5-(((tert-Butyldimethylsilyl)oxy)methyl)-6-chloro-2-methylpyrimidin-4-yl)methyl)quinolin-8-amine (**70**)*

A mixture of **69** (270 mg, 0.9 mmol), quinolin-8-amine (194 mg, 1.35 mmol), and AcOH (1 drop) in methanol (15 mL) was stirred at room temperature for 1h. Then $NaBH_3CN$ (170 mg, 2.0 mmol) was added to the reaction solution. The resulting suspension was stirred at room temperature overnight. The reaction solution was evaporated. The residue was added saturated $NaHCO_3$ aqueous solution (10 mL)

and extracted with dichloromethane (50 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by silica gel column chromatography (petroleum ether/dichloromethane = 1/1) to give the desired product (200 mg, 52%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (d, *J* = 2.8 Hz, 1H), 8.07 (d, *J* = 8.0 Hz, 1H), 7.48-7.34 (m, 3H), 7.10 (d, *J* =8.0 Hz, 1H), 6.72 (d, *J* = 7.6 Hz, 1H), 4.95 (s, 2H), 4.76 (s, 2H), 2.77 (s, 3H), 0.94 (s, 9 H), 0.17 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.8, 167.5, 160.0, 147.4, 144.3, 138.7, 136.0, 128.8, 127.8, 126.0, 121.6, 114.7, 105.2, 58.8, 46.3, 26.0, 25.9, 18.5, -5.2. MS (ESI/APCI) *m/z* 314.7 $[M\text{-}TBS+2H]^+$.

*4.1.50. (4-Chloro-2-methyl-6-((quinolin-8-ylamino)methyl)pyrimidin-5-yl)methanol (**71**)*

To the solution of **70** (190 mg, 0.44 mmol) in THF (5 mL) was added pyridine hydrofluoride (60 wt percent in pyridine, 3 mL). The resulting suspension was stirred at room temperature for 4h. The reaction solution was evaporated. The residue was added water (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by silica gel column chromatography (dichloromethane/methanol/ammonium hydroxide = 100/2/1) to give the desired product (100 mg, 72%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.11 (d, *J* = 7.6 Hz, 1H), 7.46-7.37 (m, 2H), 7.16 (d, *J* = 8.0 Hz, 1H), 6.84 (d, *J* = 6.8 Hz, 1H), 4.93 (s, 2H), 4.75 (s, 2H), 2.78 (s, 3H). MS (ESI/APCI) *m/z* 314.8 $[M+H]^+$.

*4.1.51. (4-Chloro-2-methyl-6-((methyl(quinolin-8-yl)amino)methyl)pyrimidin-5-yl)methanol (**72**)*

A mixture of **71** (40 mg, 0.13 mmol), formaldehyde (37 wt percent in water, 0.5 mL) and AcOH (1 drop) in methanol (10 mL) was stirred for 30min. $NaBH_3CN$ (62 mg, 1 mmol) was then added to the reaction solution. The resulting suspension was stirred at room temperature overnight. The reaction mixture was evaporated. The residue was added saturated $NaHCO_3$ aqueous solution (10 mL) and extracted with dichloromethane (50 mL). 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 give the desired product (31 mg, 74%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86 (d, *J* = 4.4 Hz, 1H), 8.20 (d, *J* = 8.4 Hz, 1H), 7.65-7.36 (m, 4H), 4.81 (s, 2H), 4.62 (s, 2H), 2.79 (s, 3H), 2.72 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.7, 166.9, 162.8, 148.7, 142.3, 137.6, 129.8, 129.4, 127.1, 123.5, 121.8, 119.3, 99.6, 61.2, 57.8, 43.5, 25.7. MS (ESI/APCI) *m/z* 328.8 $[M+H]^+$.

*4.1.52. (2-Methyl-4-((methyl(quinolin-8-yl)amino)methyl)-6-(4-methylpiperazin-1-yl)pyrimidin-5-yl)methanol (**73**)*

A mixture of **72** (30 mg, 0.09 mmol), TEA (91 mg, 0.9 mmol) and *N*-methylpiperazine (45 mg, 0.45 mmol) in ethanol (5 mL) was stirred at reflux overnight. The reaction mixture was concentrated to remove the solvent. The residue was purified by silica gel column chromatography (dichloromethane/methanol/ammonium hydroxide = 100/2/1) to give the desired product (28 mg, 80%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (dd, *J* = 4.0, 1.6 Hz, 1H), 8.17 (dd, *J* = 4.0, 1.6 Hz, 1H), 7.58-7.48 (m, 2H), 7.45-7.38 (m, 2H), 6.68 (t, *J* = 7.2 Hz, 1H), 4.51-4.44 (m, 4H), 3.85-3.71 (m, 4H), 2.85 (s, 3H), 2.59-2.52 (m, 7H), 2.34 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.3, 166.2, 165.2, 149.7, 148.8, 142.9, 136.9, 129.7, 127.0, 123.2, 121.5, 118.6, 117.3, 61.6, 58.0, 55.5, 49.1, 46.3, 43.5, 26.0. HRMS (ESI): calcd for $C_{22}H_{29}N_6O$ $[M+H]^+$ 393.2397, found 393.2394. Purity: 99.5%.

4.2. In vitro biological assays

4.2.1. HPB-ALL CXCR4 competitive binding assay

HPB-ALL cells were maintained in RPMI-1640 (Gibico) supplemented with 10% FBS (Hyclone). APC-conjugated anti-human CXCR4 was from Sungene. EC_{80} was first determined for 12G5 binding to

CXCR4. Then the compounds for testing (5 μL) 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 (95 μL) 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 (100 μL, final) 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. Compounds **3** and **37** were tested by at least two independent repeat experiments. Each independent experiment contained two technical replicates.

4.2.2. FLIPR Tetra calcium mobilization assay

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 (20 μL/well) 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 (10 μL/well) and CXCL12 (10 μL/well) were added to the plates. The final volume of the mixture was 60 μL/well. 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. Each compound was tested by at least two independent repeat experiments. Each independent experiment contained two technical replicates.

4.2.3. 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 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 **3** 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 (10 μL final) 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 **3** was tested by two independent repeat experiments. Each independent experiment contained two technical replicates.

4.2.4. 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 (20 μL/well) 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 (10 μL/well) and CCL20 (10 μL/well) were added to the plates. The final volume of the mixture was 60 μL/well. 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. Compound **3** was tested by two independent repeat experiments. Each independent experiment contained two technical replicates.

4.2.5. 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 in 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.

4.2.6. 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).

4.3. Preliminary in vitro safety and DMPK test

CYP inhibitory potency and liver microsomes metabolic stability were evaluated as previously reported [29]. Caco-2 and plasma protein binding assays were reported before [30].

Acknowledgements

This work was supported by National Natural Science Foundation of China (Grant No. 81973161, 81773561, 31671436 and 31830051), the Priority Academic Program Development of the Jiangsu Higher Education Institutes (PAPD) and the Jiangsu Key Laboratory of Neuropsychiatric Diseases (BM2013003).

Conflicts of interest

There are no conflicts to declare.

Appendix A. Supplementary data

Supplementary data related to this article can be found at

References

[1] A.A. Haqqani, J.C. Tilton, Entry inhibitors and their use in the treatment of HIV-1 infection, Antiviral Res. 98 (2013) 158-170.

[2] T. Lapidot, O. Kollet, The essential roles of the chemokine SDF-1 and its receptor CXCR4 in human stem cell homing and repopulation of transplanted immune-deficient NOD/SCID and

NOD/SCID/B2m(null) mice, Leukemia 16 (2002) 1992-2003.
[3] O. Kollet, I. Petit, J. Kahn, S. Samira, A. Dar, A. Peled, V. Deutsch, M. Gunetti, W. Piacibello, A. Nagler, T. Lapidot, Human CD34(+)CXCR4(-) sorted cells harbor intracellular CXCR4, which can be functionally expressed and provide NOD/SCID repopulation, Blood 100 (2002) 2778-2786.
[4] T. Pozzobon, G. Goldoni, A. Viola, B. Molon, CXCR4 signaling in health and disease, Immunol. Lett. 177 (2016) 6-15.
[5] W.T. Choi, S. Duggineni, Y. Xu, Z. Huang, J. An, Drug discovery research targeting the CXC chemokine receptor 4 (CXCR4), J. Med. Chem. 55 (2012) 977-994.
[6] B. Debnath, S. Xu, F. Grande, A. Garofalo, N. Neamati, Small molecule inhibitors of CXCR4, Theranostics 3 (2013) 47-75.
[7] Müller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verástegui E, Zlotnik A, Involvement of chemokine receptors in breast cancer metastasis, Nature 410 (2001) 50–56.
[8] Furusato B, Mohamed A, Uhlen M, Rhim JS. CXCR4 and cancer, Pathol. Int. 60 (2010) 497-505.
[9] Debarati Mukherjee, Jihe Zhao, The Role of chemokine receptor CXCR4 in breast cancer Metastasis, Am J Cancer Res. 3 (2013) 46-57.
[10] J.F. DiPersio, G.L. Uy, U. Yasothan, P. Kirkpatrick, Plerixafor, Nat. Rev. Drug Discov. 8 (2009) 105-106.
[11] E. De Clercq, AMD3100/CXCR4 inhibitor, Front. Immunol. 6 (2015) 276.
[12] N.D. Stone, S.B. Dunaway, C. Flexner, C. Tierney, G.B. Calandra, S. Becker, Y.J. Cao, I.P. Wiggins, J. Conley, R.T. MacFarland, J.G. Park, C. Lalama, S. Snyder, B. Kallungal, K.L. Klingman, C.W. Hendrix, Multiple-dose escalation study of the safety, pharmacokinetics, and biologic activity of oral AMD070, a selective CXCR4 receptor inhibitor, in human subjects, Antimicrob. Agents Chemother. 51 (2007) 2351-2358.
[13] R.T. Skerlj, G.J. Bridger, A. Kaller, E.J. McEachern, J.B. Crawford, Y. Zhou, B. Atsma, J. Langille, S. Nan, D. Veale, T. Wilson, C. Harwig, S. Hatse, K. Princen, E. De Clercq, D. Schols, Discovery of novel small molecule orally bioavailable C-X-C chemokine receptor 4 antagonists that are potent inhibitors of T-tropic (X4) HIV-1 replication, J. Med. Chem. 53 (2010) 3376-3388.
[14] K.S. Gudmundsson, P.R. Sebahar, L.D. Richardson, J.F. Miller, E.M. Turner, J.G. Catalano, A. Spaltenstein, W. Lawrence, M. Thomson, S. Jenkinson, Amine substituted N-(1H-benzimidazol-2ylmethyl)-5,6,7,8-tetrahydro-8-quinolinamines as CXCR4 antagonists with potent activity against HIV-1, Bioorg. Med. Chem. Lett. 19 (2009) 5048-5052.
[15] S. Jenkinson, M. Thomson, D. McCoy, M. Edelstein, S. Danehower, W. Lawrence, P. Wheelan, A. Spaltenstein, K. Gudmundsson, Blockade of X4-tropic HIV-1 cellular entry by GSK812397, a potent noncompetitive CXCR4 receptor antagonist, Antimicrob. Agents Chemother. 54 (2010) 817-824.
[16] V.M. Truax, H. Zhao, B.M. Katzman, A.R. Prosser, A.A. Alcaraz, M.T. Saindane, R.B. Howard, D. Culver, R.F. Arrendale, P.R. Gruddanti, T.J. Evers, M.G. Natchus, J.P. Snyder, D.C. Liotta, L.J. Wilson, Discovery of tetrahydroisoquinoline-based CXCR4 antagonists, ACS Med. Chem. Lett. 4 (2013) 1025-1030.
[17] Z. Liang, W. Zhan, A. Zhu, Y. Yoon, S. Lin, M. Sasaki, J.M. Klapproth, H. Yang, H.E. Grossniklaus, J. Xu, M. Rojas, R.J. Voll, M.M. Goodman, R.F. Arrendale, J. Liu, C.C. Yun, J.P. Snyder, D.C. Liotta, H. Shim, Development of a unique small molecule modulator of CXCR4, PLOS One 7 (2012) e34038.
[18] R. Wu, W. Yu, C. Yao, Z. Liang, Y. Yoon, Y. Xie, H.Shim, R.Bai, Amide-sulfamide modulators as effective anti-tumor metastatic agents targeting CXCR4/CXCL12 axis, Eur. J. Med. Chem. 185

(2020) 111823.
[19] T. Murakami, S. Kumakura, T. Yamazaki, R. Tanaka, M. Hamatake, K. Okuma, W. Huang, J. Toma, J. Komano, M. Yanaka, Y. Tanaka, N. Yamamoto, The novel CXCR4 antagonist KRH-3955 is an orally bioavailable and extremely potent inhibitor of human immunodeficiency virus type 1 infection: comparative studies with AMD3100, Antimicrob. Agents Chemother. 53 (2009) 2940-2948.
[20] G. Thoma, M.B. Streiff, J. Kovarik, F. Glickman, T. Wagner, C. Beerli, H.G. Zerwes, Orally bioavailable isothioureas block function of the chemokine receptor CXCR4 in vitro and in vivo, J. Med. Chem. 51 (2008) 7915-7920.
[21] Z. Li, Y. Wang, C. Fu, X. Wang, J.J. Wang, Y. Zhang, D. Zhou, Y. Zhao, L. Luo, H. Ma, W. Lu, J. Zheng, X. Zhang, Design, synthesis, and structure-activity-relationship of a novel series of CXCR4 antagonists, Eur. J. Med. Chem. 149 (2018) 30-44.
[22] F. Zhu, Y. Wang, Q. Du, W. Ge, Z. Li, X. Wang, C.Fu, L. Luo, S. Tian, H. Ma, J. Zheng, Y. Zhang, X. Sun, S. He, X. Zhang, Structural optimization of aminopyrimidine-based CXCR4 antagonists, Eur. J. Med. Chem. 187 (2020) 111914.
[23] J.A. Secrist, C.J. Hickey, R.E. Norris, A convenient total synthesis of (.+-.)-(7E,9E)-trisporic acid B methyl ester, J. Org. Chem. 42 (1977) 525-527.
[24] R.C. Reid, M. Yau, R. Singh, J. Lim, D.P. Fairlie, Stereoelectronic Effects Dictate Molecular Conformation and Biological Function of Heterocyclic Amides, J. Am. Chem. Soc. 136 (2014) 11914-11917.
[25] K. Princen, S. Hatse, K. Vermeire, E. De Clercq, D. Schols, Evaluation of SDF-1/CXCR4-induced Ca2+ signaling by fluorometric imaging plate reader (FLIPR) and flow cytometry, Cytometry A 51 (2003) 35-45.
[26] A. Van Hout, T. D'Huys, M. Oeyen, D. Schols, T. Van Loy, Comparison of cell-based assays for the identification and evaluation of competitive CXCR4 inhibitors, PLOS One 12 (2017) e0176057.
[27] K.S. Gudmundsson, S.D. Boggs, J.G. Catalano, A. Svolto, A. Spaltenstein, M. Thomson, P. Wheelan, S. Jenkinson, Imidazopyridine-5,6,7,8-tetrahydro-8-quinolinamine derivatives with potent activity against HIV-1, Bioorg. Med. Chem. Lett. 19 (2009) 6399-6403.
[28] K.S. Gudmundsson, P.R. Sebahar, L.D. Richardson, J.F. Miller, E.M. Turner, J.G. Catalano, A. Spaltenstein, W. Lawrence, M. Thomson, S. Jenkinson, Amine substituted N-(1H-benzimidazol-2ylmethyl)-5,6,7,8-tetrahydro-8-quinolinamines as CXCR4 antagonists with potent activity against HIV-1, Bioorg. Med. Chem. Lett. 19 (2009) 5048-5052.
[29] Y. Dong, K. Li, Z. Xu, H. Ma, J. Zheng, Z. Hu, S. He, Y. Wu, Z. Sun, L. Luo, J. Li, H. Zhang, X. Zhang, Exploration of the linkage elements of porcupine antagonists led to potent Wnt signaling pathway inhibitors, Bioorg. Med. Chem. 23 (2015) 6855-6868.
[30] W. Lu, Y. Liu, H. Ma, J. Zheng, S. Tian, Z. Sun, L. Luo, J. Li, H. Zhang, Z.J. Yang, X. Zhang, Design, Synthesis, and Structure-Activity Relationship of Tetrahydropyrido[4,3-d]pyrimidine Derivatives as Potent Smoothened Antagonists with in Vivo Activity, ACS Chem. Neurosci. 8 (2017) 1980-1994.

Declaration of interests

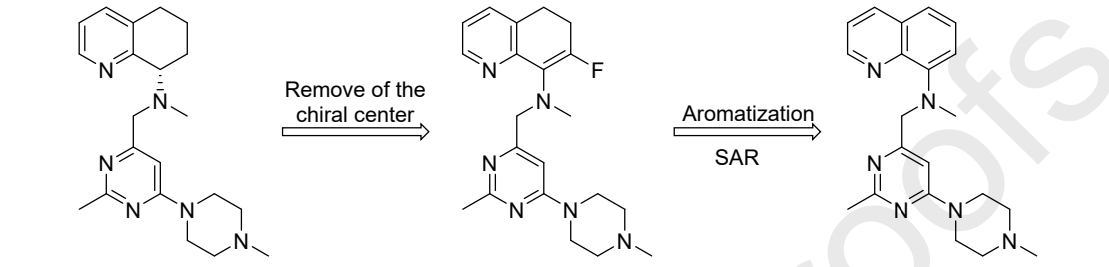
☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical Abstract

Design, Synthesis, and Evaluation of Novel CXCR4 Antagonists Based on an Aminoquinoline Template

Yu Lin, Zhanhui Li, Chen Xu, Kaijiang Xia, Shuwei Wu, Yongjin Hao, Qing Yang, Haikuo Ma, Jiyue Zheng, Lusong Luo, Fang Zhu, Sudan He, and Xiaohu Zhang



1

binding IC_{50} = 8.8 ± 1.0 nM
Ca^{2+} Flux IC_{50} = 0.021 ± 0.004 nM
clogP = 2.06
pKa = 7.2

2

binding IC_{50} = 15 ± 3.5 nM
Ca^{2+} Flux IC_{50} = 1.3 ± 0.4 nM
clogP = 2.07
pKa = 6.9

3

binding assay IC_{50} = 57 ± 8.0 nM
Ca^{2+} Flux IC_{50} = 0.24 ± 0.01 nM
clogP = 2.13
pKa = 7.0

Aminoquinoline has been identified as a novel scaffold for CXCR4 antagonists. The synthesis is greatly simplified compares with classical tetrahydroquinoline, which contains a chiral center.

Highlights

(1) Aminoquinoline is identified as a novel scaffold for CXCR4 antagonists.

(2) The synthesis is greatly simplified compares with classical tetrahydroquinoline.

(3) Compound **3** competes with APC-conjugate 12G5 for CXCR4 binding (IC_{50} = 57 nM).

(4) Compound **3** inhibits CXCL12 induced cytosolic calcium increase (IC_{50} = 0.24 nM).

(5) Compound **3** inhibits CXCR4/CXLC12 mediated chemotaxis in a transwell assay.