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Design, synthesis, and evaluation of pyrrolidine based CXCR4 antagonists with *in vivo* anti-tumor metastatic activity

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Abstract: The chemokine receptor CXCR4 has been proposed as a drug target based on its important functions in HIV infection, inflammation/autoimmune diseases and cancer metastasis. Herein we report the design, synthesis and evaluation of novel CXCR4 antagonists based on a pyrrolidine scaffold. The structural exploration/optimization identified numerous potent CXCR4 antagonists, represented by compound **46**, which displayed potent binding affinity to CXCR4 receptor (IC_{50} = 79 nM competitively displacing fluorescent 12G5 antibody) and inhibited CXCL12 induced cytosolic calcium flux (IC_{50} = 0.25 nM). Moreover, in a transwell invasion assay, compound **46** significantly mitigated CXLC12/CXCR4 mediated cell migration. Compound **46** exhibited good physicochemical properties (MW 367, $logD_{7.4}$ 1.12, pKa 8.2) and excellent *in vitro* safety profiles (e.g., hERG patch clamp IC_{50} > 30 μM and minimal CYP isozyme inhibition). Importantly, **46** displayed much improved metabolic stability in human and rat liver microsomes. Lastly, **46** demonstrated marked efficacy in a cancer metastasis model in mice. These results strongly support **46** as a prototypical lead for the development of promising CXCR4 antagonists as clinical candidates.

Keywords: antagonist, chemokine, CXCL12, CXCR4, GPCR

Abbreviations: APC, allophycocyanin; CCD, charge coupled device; CYP, cytochrome P450; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethylamine; DMEM, Dulbecco's modified eagle medium; DMSO, dimethyl sulfoxide; DPBS, Dulbecco's phosphate buffered saline; EMCCD, electron-multiplying CCD; FACS, fluorescence activated cell sorter; FBS, fetal bovine serum; FLIPR, fluorescent imaging plate reader; GPCR, G protein-coupled receptor; HBSS, Hank's balanced salt solution; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; hERG, human ether-a-go-go-related gene; HI-FBS, heat inactivated FBS; HIV, human immunodeficiency virus; HLM, h

uman liver microsomes; HPLC, high performance liquid chromatography; ICCD, intensified CC D; IPA, isopropanol; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PFA, pa raformaldehyde; RFP, red fluorescence protein; RLM, rat liver microsomes; RPMI, roswell park memorial institute; rt, room temperature; SAR, structure-activity-relationship; SDF-1, stromal cel l-derived factor-1; SEM, standard error measurement; TEA, triethylamine; TFA, trifluoroacetic a cid; THF, tetrahydrofuran; THQ, tetrahydroquinoline; TIQ, tetrahydroisoquinoline; TsOH, *p*-tolue nesulfonic acid.

1. Introduction

CXCR4 is a chemokine receptor which belongs to the G protein-coupled receptor super family. CXCR4 and its natural ligand CXCL12 (also known as stromal cell-derived factor-1, or SDF-1) regulate an array of important physiological functions such as chemotaxis, hematopoietic stem cell homing and retention [1, 2]. Dysregulation of CXCR4/CXCL12 axis has been linked to human pathological conditions (e.g., HIV virus infection, inflammatory/autoimmune diseases and cancer metastasis) [3, 4]. Consequently, development of CXCR4 antagonists to modulate the pathological functions of CXCL12/CXCR4 axis has been proposed as an important strategy for the treatment of HIV infection, inflammatory diseases and cancer [5-9].

CXCR4 was first identified as a coreceptor used by T cell-tropic (X4) HIV-1 virus to gain cellular entry [10]. As a result, the majority of early research of CXCR4 antagonists focused on anti-HIV application. This effort culminated in the identification and development of AMD3100 (plerixafor, Figure 1) [11, 12], the most advanced small molecule CXCR4 antagonist. Unfortunately, dose-limiting toxicity and poor oral bioavailability impeded its application as an orally administered daily drug for chronic HIV treatment. During clinical trials, however, AMD3100 surprisingly displayed strong effect on mobilizing hematopoietic stem cell. Consequently, AMD3100 was approved by the FDA for the treatment of non-Hodgkin's lymphoma and multiple myeloma patients with autologous stem cell transplants. This unexpected clinical utilization of AMD3100 significantly expanded the scope of CXCR4 research [13]. More recent discoveries of CXCR4/CXCL12 function include cancer cell proliferation, angiogenesis, and metastasis. In addition, CXCR4 was reported to modulate cancer immunity by trafficking of key immune cells into tumor microenvironment. Since many of the immune cells were immunosuppressive (e.g., Treg, myeloid derived suppressor cells) rather than immunoreactive (e.g., $CD8^+$ effector T lymphocytes), CXCL12/CXCR4 axis plays an important role in evasion of immune surveillance [4]. The dose-limiting toxicity and poor oral bioavailability of AMD3100, combined with the emerging evidence of CXCR4/CXCL12 in modulating cancer cell proliferation, metastasis and immunity at the tumor microenvironment, sparked significant renewed interests to discover new CXCR4 antagonists with high potency, low toxicity, and acceptable oral bioavailability.

In order to address the poor oral bioavailability associated with AMD3100, systemic effort aimed to reduce molecular weight and cationic charges was carried out [14-24]. These exercises led to the identification of AMD11070 [17, 18, 25]. AMD11070 displayed improved bioavailability and was progressed into clinical investigation. However, AMD11070 moderately inhibited CYP450 isozymes (e.g., CYP3A4, 2D6), likely due to the benzimidazole moiety presented in the molecule. Further

structural modification to replace this functional group resulted in the identification of GSK812397 [24, 26] and TIQ15 [23, 27] (Figure 1).

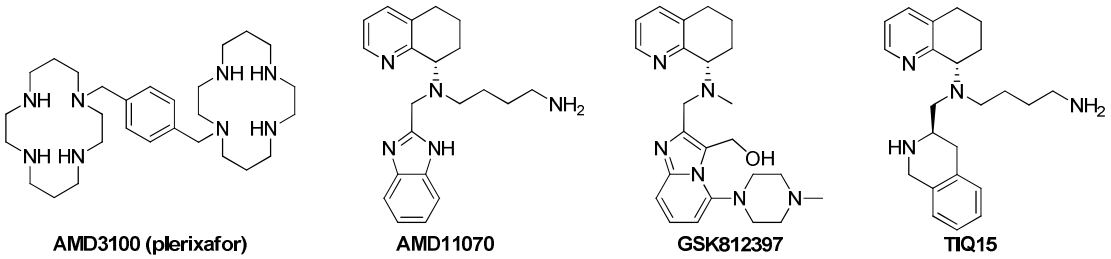
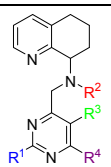


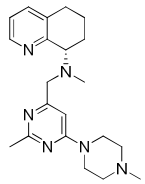
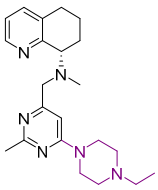
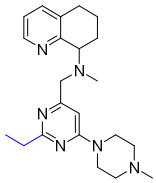
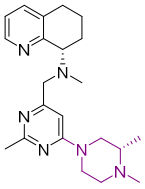
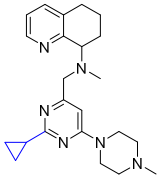
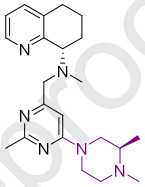
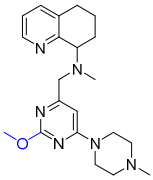
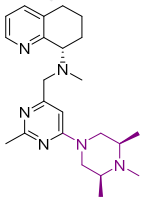
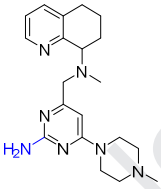
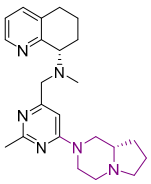
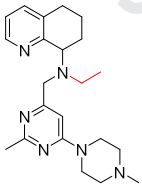
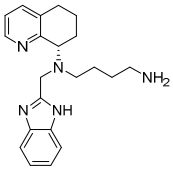
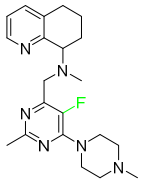
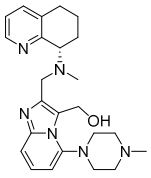
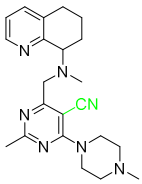
Fig. 1. Representatives of small molecule CXCR4 antagonists reported in the literature.

Taking a similar approach, we have developed and reported a series of CXCR4 antagonists based on aminopyrimidine central core structure (exemplified by compound **1** in Figure 2) [28, 29]. These compounds competitively displace 12G5 antibody, inhibit CXCL12 induced cytosolic calcium flux, and mitigate CXCL12 induced cell migration in a transwell invasion assay. Moreover, they generally show good physicochemical properties and safety profiles (moderate to low hERG and CYP isozyme inhibition). However, an outstanding issue related to these compounds is the fast clearance in liver microsomes, which could result in poor oral bioavailability and short half-life. In order to address this problem, we did an extensive structure-metabolic relationship study (Table 1). For example, the 2-position (R^1) of the central pyrimidine ring was explored by substitutions with increased size and lipophilicity (compounds **2** and **3**) or with increased polarity and hydrophilicity (compounds **4** and **5**). Unfortunately, the metabolic stability of these compounds was unanimously poor in rat liver microsomes. Similar results were obtained for R^2, R^3, and R^4 variants. Since all these changes at R^1-R^4 had no impact to alter the metabolic stability, we posited that the tetrahydroquinoline (THQ) was likely the culprit for high clearance (the only unchanged structural element in this exercise). This hypothesis was supported by the metabolic stability data of AMD11070 and GSK812397 (Table 1), both of which contain a THQ tethered to a distinctive heterocyclic group. Direct evidence concerning the metabolic liability of the THQ functionality was presented by scientists from Emory University based on data of their tetrahydroisoquinoline (TIQ) series of compounds [27]. They showed that hydroxylation on the THQ moiety in mouse liver microsomes was responsible for high clearance. Blockade of the metabolic soft spot on the THQ moiety shifted the metabolic liability to the TIQ moiety and ring opening of the THQ moiety had helped to improve metabolic stability. Encouraged by these results, we formulated a structural modification plan as described in Figure 2. Herein we report the design, synthesis and evaluation of novel CXCR4 antagonists based on a pyrrolidine scaffold that showed improved metabolic stability in human and rat liver microsomes. Furthermore, compound **46** demonstrated marked efficacy in a mouse cancer metastasis model.

Table 1. Metabolic stability of compounds **1-13**, AMD11070, and GSK812397



Cmpd	Structure	RLM[a]	Cmpd	Structure	RLM[a]

		Cl_{int} (mL/min/kg)	$t_{1/2}$ (min)			Cl_{int} (mL/min/kg)	$t_{1/2}$ (min)
1		1567	1.59	**9**		2179	1.14
2		2092	1.19	**10**		1549	1.60
3		2439	1.02	**11**		>497	<1
4		1129	2.20	**12**		>497	<1
5		767	3.24	**13**		>497	<1
6		1275	1.95	**AMD 11070**		1776	1.40
7		1077	2.31	**GSK 812397**		651	3.82
8		1644	1.51				

[a] All the compounds were tested at 1 μM concentration. RLM = Rat liver microsomes.

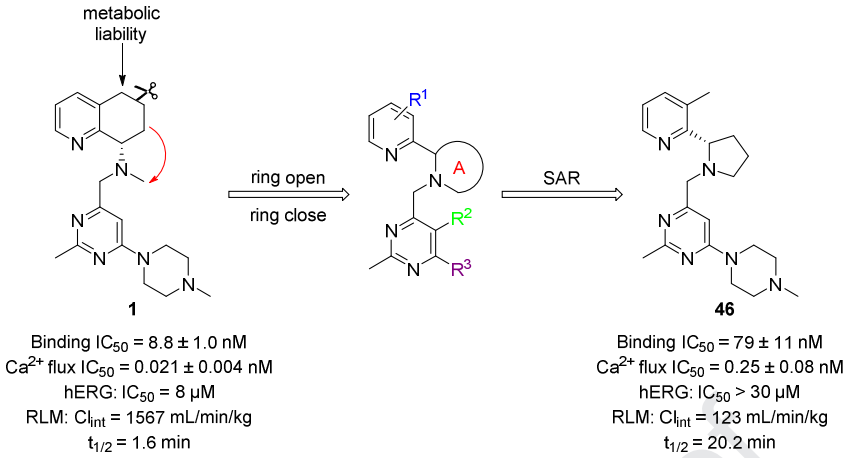


Fig. 2. Proposed SAR study plan.

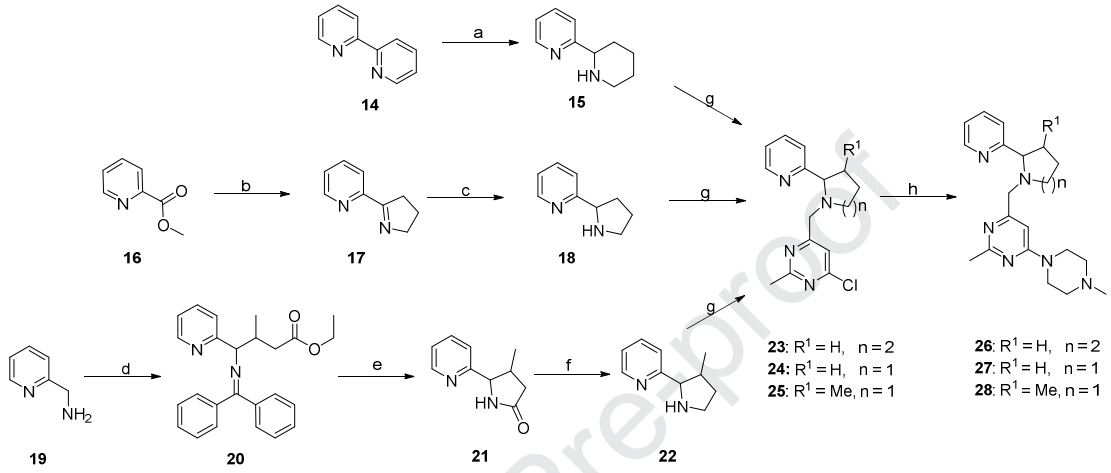
2. Chemistry

The synthesis of compounds **26-28** was performed as shown in Scheme 1. Intermediate **15** was synthesized by reduction of commercially available **14** with tin powder followed by Pd/C. Intermediate **17** was synthesized by cyclization of commercially available **16** [30]. Reduction of **17** with $NaBH_4$ yielded intermediate **18**. Commercially available **19** and diphenyl ketone were reacted in toluene to give imine intermediate, which was then reacted with ethyl (*E*)-but-2-enoate to afford **20** under alkaline conditions. Cyclization of **20** in the presence of concentrated HCl afforded **21**, which was then reduced by $LiAlH_4$ in THF to give intermediate **22**. Compounds **15**, **18**, and **22** were reacted with 4-chloro-6-(chloromethyl)-2-methylpyrimidine [29] in the presence of DIPEA and KI to give intermediates **23-25**. The syntheses of compounds **26-28** were achieved by reacting intermediates **23-25** with *N*-methylpiperazine in EtOH.

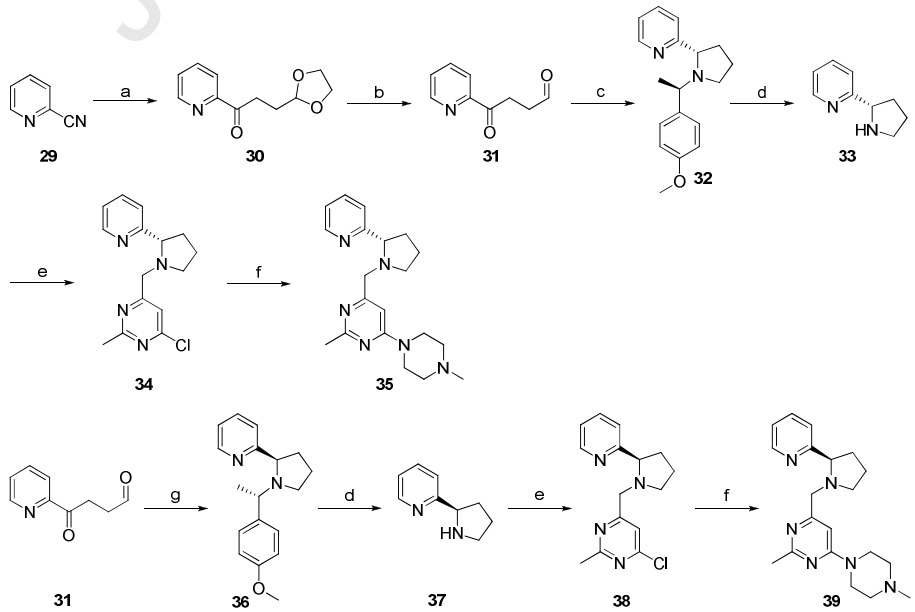
The synthesis of compounds **35** and **39** was performed as shown in Scheme 2. Commercially available 2-(2-bromoethyl)-1,3-dioxolane was reacted with Mg in THF to give Grignard reagent, which was reacted with commercially available **29** to give **30**. Deprotection of **30** with 3 N HCl in acetone afforded **31**, which was reacted with (R)-1-(4-methoxyphenyl)ethan-1-amine in the presence of $NaBH(OAc)_3$ to give **32** [31]. Debenzylation of **32** with TFA provided **33**, which was reacted with 4-chloro-6-(chloromethyl)-2-methylpyrimidine [29] in the presence of DIPEA and KI to give **34**. Compound **35** was achieved by reacting intermediate **33** with *N*-methylpiperazine in EtOH. Similarly, compound **39** was synthesized by using (S)-1-(4-methoxyphenyl)ethan-1-amine in place of (R)-1-(4-methoxyphenyl)ethan-1-amine.

The synthesis of compounds **46-49**, **50**, and **52-55** was performed as shown in Scheme 3. Commercially available 2-(2-bromoethyl)-1,3-dioxolane was reacted with Mg in THF to give Grignard reagent, which was reacted with commercially available **40a-d** to give **41a-d**. Deprotection of **41a-d** with 3 N HCl in acetone afforded **42a-d**, which were reacted with (R)-1-(4-methoxyphenyl)ethan-1-amine in the presence of $NaBH(OAc)_3$ to give **43a-d**. Debenzylation of **43a-d** with TFA provided **44a-d**, which were reacted respectively with 4-chloro-6-(chloromethyl)-2-methylpyrimidine [29] in the presence of DIPEA and KI to give **45a-d.** Compounds **46-49** were achieved by reacting intermediates **45a-d** with *N*-methylpiperazine in EtOH. Compounds **53-55** were achieved by reacting intermediate **45a** with *N*-methyl-1,4-diazepane,

N,*N*-dimethylpiperidin-4-amine, and 2-morpholinoethan-1-amine, respectively. Compound **50** was achieved by reacting intermediate **44a** with 5-fluoro-4-(fluoromethyl)-2-methyl-6-(4-methylpiperazin-1-yl)pyrimidine [29] in the presence of 2-ethyl-2-(hydroxymethyl)propane-1,3-diol and KI [32]. Intermediate **44a** was reacted with *tert*-butyl 4-(6-(chloromethyl)-5-cyano-2-methylpyrimidin-4-yl)piperazine-1-carboxylate [29] in the presence of DIPEA and KI to give intermediate **51**. Deprotection of BOC group followed by reaction with HCHO/$NaBH_3CN$ yielded compound **52**.

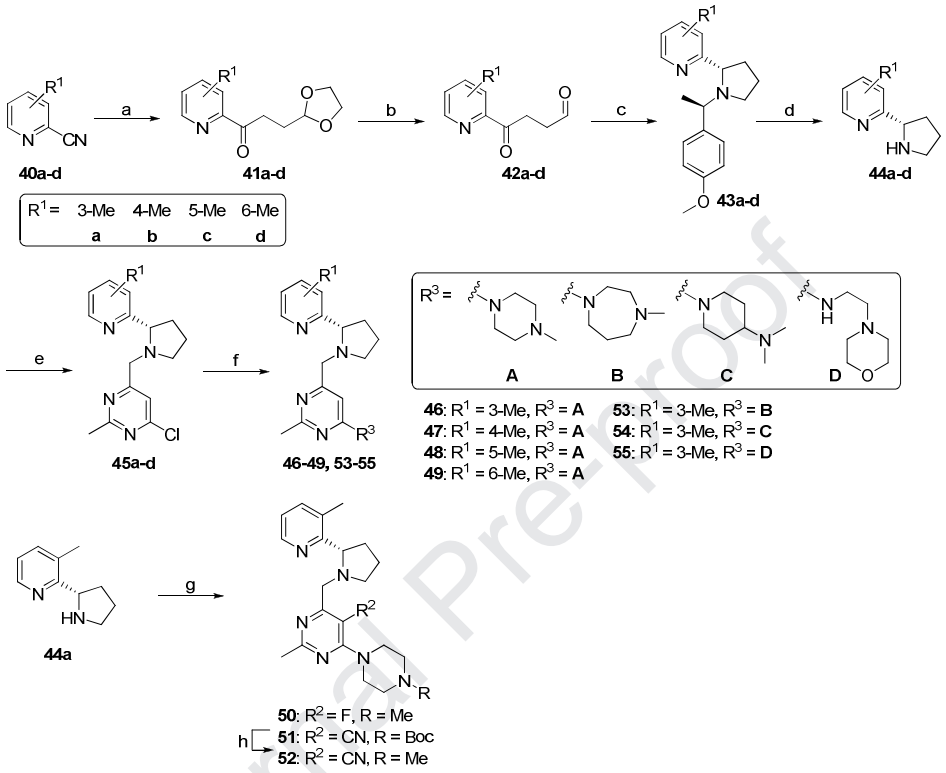


Scheme 1. Reagents and conditions: (a) 1) Sn, 6 N HCl (aq.), EtOH, 100 ^oC, 12 h; 2) Pd/C, H_2, MeOH, rt, 16 h; (b) 1) 1-vinyl pyrrolidin-2-one, NaH, THF, 60 ^oC, 2 h; 2) 4 N HCl (aq.), rt, overnight; (c) $NaBH_4$, acetic acid, MeOH, -20 ^oC, 3 h; (d) 1) diphenyl ketone, TsOH, toluene, 120 ^oC, overnight; 2) ethyl (*E*)-but-2-enoate, benzyltriethylammonium chloride, 50% NaOH (aq.), MeCN, rt, overnight; (e) conc. HCl, MeCN, rt, 2 h; (f) $LiAlH_4$, THF, 50 ^oC, overnight; (g) 4-chloro-6-(chloromethyl)-2-methylpyrimidine, DIPEA, KI, MeCN, rt, overnight; (h) *N*-methylpiperazine, TEA, EtOH, 80 ^oC,3 h.



Scheme 2. Reagents and conditions: (a) 1) 2-(2-bromoethyl)-1,3-dioxolane, Mg, iodine, THF, 30 ^oC, 1

h; 2) **29**, THF, 0 °C; 3 h; (b) 3 N HCl (aq.), acetone, rt, overnight; (c) (R)-1-(4-methoxyphenyl)ethan-1-amine, $NaBH(OAc)_3$, DCM, -70 °C to rt, overnight; (d) TFA, 50 °C, 12 h; (e) 4-chloro-6-(chloromethyl)-2-methylpyrimidine, DIPEA, KI, MeCN, rt, overnight; (f) *N*-methylpiperazine, TEA, EtOH, 80 °C, 3 h; (g) (S)-1-(4-methoxyphenyl)ethan-1-amine, $NaBH(OAc)_3$, DCM, -70 °C to rt, overnight.



Scheme 3. Reagents and conditions: (a) 1) 2-(2-bromoethyl)-1,3-dioxolane, Mg, iodine, THF, 30 °C, 1 h; 2) **40a-d**, THF, 0 °C; 3 h; (b) 3 N HCl (aq.), acetone, rt, overnight; (c) (R)-1-(4-methoxyphenyl)ethan-1-amine, $NaBH(OAc)_3$, DCM, -70 °C to rt, overnight; (d) TFA, 50 °C, 12 h; (e) 4-chloro-6-(chloromethyl)-2-methylpyrimidine, DIPEA, KI, MeCN, rt, overnight; (f) corresponding amine, TEA, EtOH, 80 °C, 3 h; (g) for **50**: 5-fluoro-4-(fluoromethyl)-2-methyl-6-(4-methylpiperazin-1-yl)pyrimidine, 2-ethyl-2-(hydroxymethyl)propane-1,3-diol, H_2O, KI, 120 °C, overnight; for **51**: *tert*-butyl 4-(6-(chloromethyl)-5-cyano-2-methylpyrimidin-4-yl)piperazine-1-carboxylate, DIPEA, KI, MeCN, rt, overnight; (h) 1) TFA, DCM, rt, 2 h; 2) $HCHO/H_2O$, $NaBH_3CN$, MeOH, rt, 2 h.

3. Results and discussion

3.1. *Structure-activity-relationship of synthesized compounds*

In our previous publications [28, 29], we used the cell based FACS 12G5 competitive binding assay as the primary screening assay. Once an active compound was identified, the compound was subjected to a FLIPR Tetra based cytosolic calcium flux assay. In this report, we used both assays to characterize our compounds and we are in a transition to switch the primary screening assay from the 12G5 competitively binding assay to the FLIPR Tetra based calcium flux functional assay. This change is based on numerous considerations. For example, competitive ligand binding assays convey no

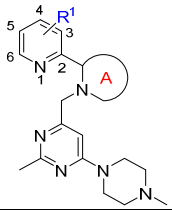
functional information while calcium flux assay provides information regarding receptor activation, potentiation, and inhibition. Competitive ligand binding assays may also miss allosteric binders which do not compete for the same binding pocket [33]. More importantly, based on our experience, the results from the 12G5 competitive binding assay correlate very well with those from the functional calcium flux assay. Moreover, the expense of the calcium flux assay was lower and the throughput was higher compared with the 12G5 competitive binding assay. Based on these assessments, we decided to use the FLIPR Tetra based calcium flux functional assay as the screening assay and the 12G5 competitively binding assay as the confirmation assay. The structure-activity-relationship is summarized in Tables 2 and 3.

Controlling key physicochemical properties in a desirable range (e.g., pKa 6-8, clogP 0-3) has been one of our strategies in optimization of CXCR4 antagonists. Here we report the pKa and clogP alongside of the biological activity of synthesized analogues. Compounds **26** and **27** are respectively formed from the ring opening/ring closure of compound **1** (Figure 2) to form piperidine or pyrrolidine as the scaffold structures. Compound **27** (319 nM) was twice as potent as **26** (620 nM) in the 12G5 binding assay. In addition, compound **27** has a lower clogP than **26**. Therefore, we chose to conduct further exploration based on compound **27**. Addition of a methyl group on the pyrrolidine ring (compound **28**) led to five folds binding affinity decrease compared with **27**. Since compound **27** contains a chiral center, we synthesized chirally pure (S)-stereoisomer **35** (220 nM) and (R)-stereoisomer **39** (> 10000 nM). As expected, compound **35** showed more potent binding compared with the racemate **27**, while its enantiomer **39** was virtually inactive. These results are consistent with our previous research and literature reports [28, 29]. Therefore, all of our subsequent compounds are synthesized to be (S)-stereoisomers. In order to explore substitution effects on the pyridine ring, we introduced methyl group at the 3, 4, 5, and 6 positions of pyridine to obtain compounds **46-49**. Among them, the 3-methyl-substituted compound **46** (79 nM) displayed significantly higher binding affinity than the other substituted analogues. In addition, by comparing the performance of compounds **46** and **35** in binding assay, we found that the 3-methyl substitution of the pyridine ring can increase binding affinity by about three folds. We also tested the functional potencies in the FLIPR Tetra based calcium mobilization assay of some compounds (**35**, **46**, **48**), and the results were largely consistent with the binding affinity assay. AMD3100 was used as the positive control.

Using compound **46** as the prototype, we continued to explore R^2 and R^3 substituents. The results are shown in Table 3. Highly basic functional group may lead to safety issues such as hERG potassium channel inhibition, CYP enzyme inhibition and phospholipidosis [34], we therefore replace the hydrogen at the R^2 position with fluorine (compound **50**) and cyano (compound **52**) as a way to mitigate overall basicity. Compared to compound **46** (pKa: 7.3), the pKa of the two compounds (**50** pKa: 6.3, **52** pKa: 5.6) were significantly reduced. However, the functional potency of compounds **50** and **52** was decreased by 4 and 7 folds compared to that of compound **46**, respectively. A basic center on the R^3 position was reported to be essential for CXCR4 antagonist activity [28, 29]. We therefore designed and synthesized compounds **53-55** with varied pKa (7.0-9.0). In addition, this small set of amines offered structural diversity such as ring expansion (compound **53**), exocyclic basic center (compound **54**), and basic center with less restriction (compound **55**). Unfortunately, compounds **54** (13 nM) and **55** (2.7 nM) displayed significantly decreased functional potency compared with compound **46**. Compound **53** (0.40 nM) showed comparable functional potency to compound **46**, but with significantly increased basicity (pKa: 8.9). Overall, compound **46** remained to be the most attractive lead with balanced potency and physicochemical properties. We carefully controlled clogP

and pKa of our compounds throughout the optimization process. Although calculated data provide efficiency and guidelines, they do not always reflect the actual values. We therefore experimentally determined the $logD_{7.4}$ (shake flask) and pKa (titration) of compound **46** (Table 3). The actual pKa (8.2) of compound **46** was almost a unit higher compared with the calculated pKa (7.3). This discrepancy was accurately reflected on actual $logD_{7.4}$ (1.12) compared with the calculated clogP (2.08). The relatively large variations between the actual and calculated data demonstrated the necessity to experimentally determine the physicochemical properties of key compounds.

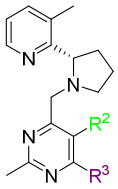
Table 2. SAR for ring A and R^1

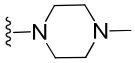
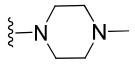
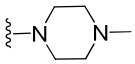
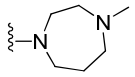
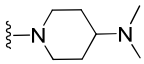
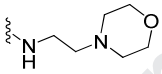


Cmpd	Ring A	R^1	Binding IC_{50} (nM)[a]	Ca^{2+} flux IC_{50} (nM)[b]	clogP[c]	pKa[d]	Cmpd	Ring A	R^1	Binding IC_{50} (nM)[a]	Ca^{2+} flux IC_{50} (nM)[b]	clogP[c]	pKa[d]
26		H	620 ± 191	ND[e]	1.80	7.8	**46**		3-Me	79 ± 11	0.25 ± 0.08	2.08	7.3
27		H	319 ± 153	ND	1.29	7.3	**47**		4-Me	216 ± 11	ND	2.08	7.3
28		H	1546 ± 342	ND	1.77	7.3	**48**		5-Me	278 ± 1.3	4.9 ± 1.0	1.75	7.3
35		H	220 ± 76	2.7 ± 0.006	1.29	7.3	**49**		6-Me	2391 ± 108	ND	1.35	7.3
39		H	>10000	ND	1.29	7.3	**AMD 3100**			561 ± 27	23 ± 2.3	-1.04	10.6

[a] Inhibition of luminescence signaling in HPB-ALL CXCR4 competitive binding assay. Data are expressed as geometric mean values of at least two runs ± the standard error measurement (SEM). [b] Inhibition of fluorescence signaling in calcium flux assay based on FLIPR Tetra. Data are expressed as geometric mean values of at least two runs ± the standard error measurement (SEM). [c] Calculated by Molinspiration. [d] Calculated by ACD/Labs 6.0. [e] ND = not determined.

Table 3. SAR for R^2 and R^3



Cmpd	R^2	R^3	Ca^{2+} flux IC_{50} (nM)[a]	clogP[b]	pKa[c]
46	H		0.25 ± 0.08	2.08[b] 1.12[d]	7.3[c] 8.2[e]
50	F		1.1 ± 0.06	2.17	6.3
52	CN		1.8 ± 0.5	1.76	5.6
53	H		0.40 ± 0.18	2.35	8.9
54	H		13 ± 6.6	2.61	9.0
55	H		2.7 ± 0.2	1.82	7.0
AMD3100			23 ± 2.3	-1.04	10.6

[a] Inhibition of fluorescence signaling in calcium flux assay based on FLIPR Tetra. Data are expressed as geometric mean values of at least two runs ± the standard error measurement (SEM). [b] Calculated by Molinspiration. [c] Calculated by ACD/Labs 6.0. [d] $logD_{7.4}$ was determined by shake flask technique. [e] pKa was determined by titration using the Sirius T3 system.

3.2. *Preliminary selectivity assessment and functional evaluation of lead compound **46***

Having established compound **46** as a promising CXCR4 antagonist (displacement of 12G5, IC_{50} = 74 nM; inhibition of CXCL12 mediated cytosolic calcium flux, IC_{50} = 0.25 nM), we next assessed the preliminary functional specificities of compound **46**. CXCR1 was selected as a representative within the CXCR sub-family and CCR6 was selected as a representative outside of the CXCR sub-family. Compound **46** was tested in the corresponding functional assays. Compound **46** was shown to be inactive up to 10,000 nM (Figure 3b, c).

CXCR4 is a chemokine receptor. One of its hallmark functions is migration and invasion of the CXCR4 expressing cells toward CXCL12 gradient. Thus, we used the Matrigel invasion assay to evaluate the effect of compound **46** on CXCL12/CXCR4 mediated cell migration. In this experiment, CXCR4 expressing MDA-MB-231 cells and compound **46** (100 nM) were added to the upper chamber. Chemoattractant CXCL12 was added to the lower chamber. AMD3100 was used as a positive control. The inhibition of cell invasion with compound **46** and AMD3100 was calculated by comparing to the cell invasion without treatment. In consistent with the 12G5 binding and the calcium mobilization data, compound **46** was more potent in the Matrigel invasion assay than AMD3100 (Figure 4). General cellular toxicity can impede cell mobility, resulting in false positive effect in cell migration assay. To exclude this possibility, Cell viability assay (ATP-based Cell Titer-Glo Luminescent Cell Viability Assay) was performed to evaluate the cytotoxicity effect of compound **46**. As shown in Figure 4c, compound **46** (up to 1000 nM) did not inhibit proliferation of multiple murine and human cells.

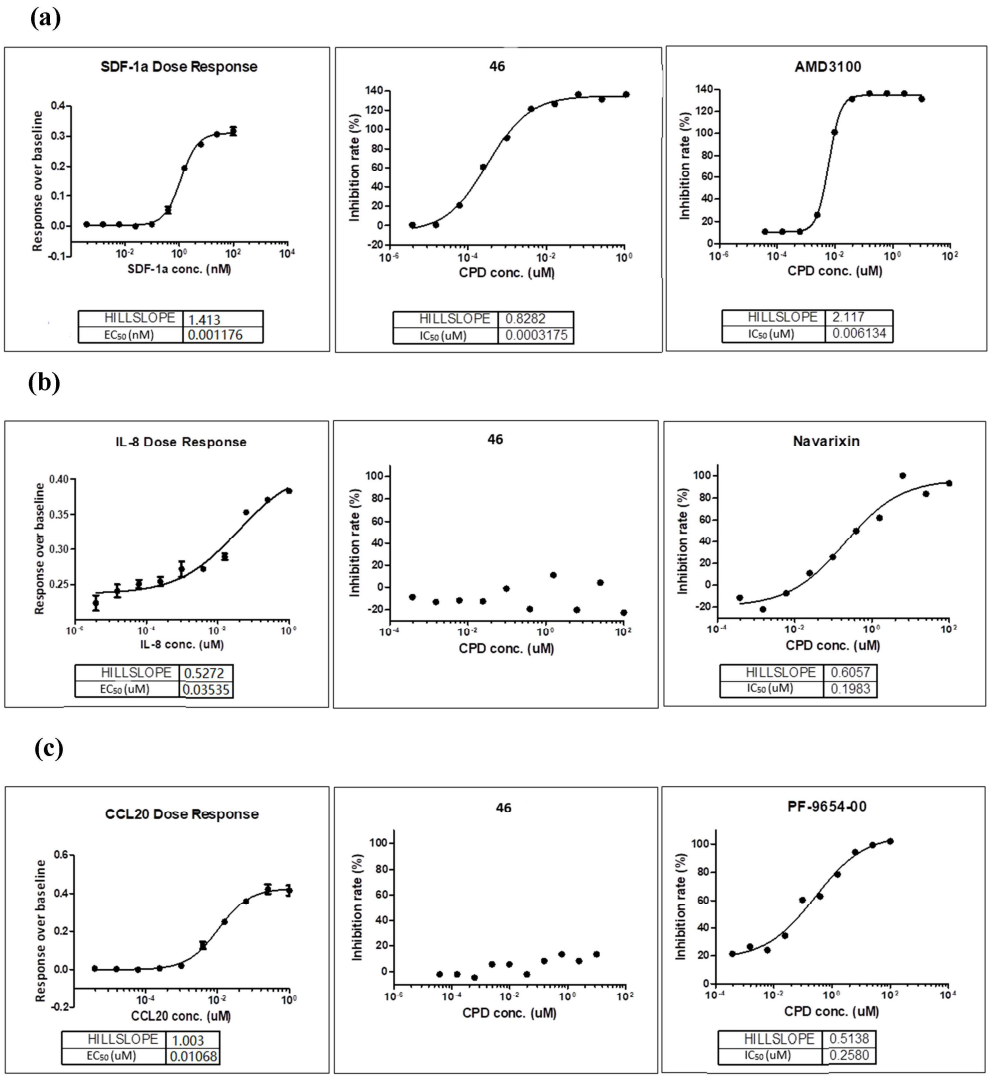


Fig. 3. Compound **46** is a potent CXCR4 antagonist, but is inactive to CXCR1 and CCR6. (a) CXCR4 dose response curve for SDF-1 (CXCL12) stimulation and inhibition curves of compound **46**/AMD3100 (positive control for CXCR4) based on a calcium flux assay. (b) CXCR1 dose response curve for IL-8 stimulation and inhibition curves of compound **46**/Navarixin (positive control for CXCR1) based on a functional cAMP assay. (c) CCR6 dose response curve for CCL20 stimulation and inhibition curves of compound **46**/PF-9654-00 (positive control for CCR6) based on a calcium flux assay.

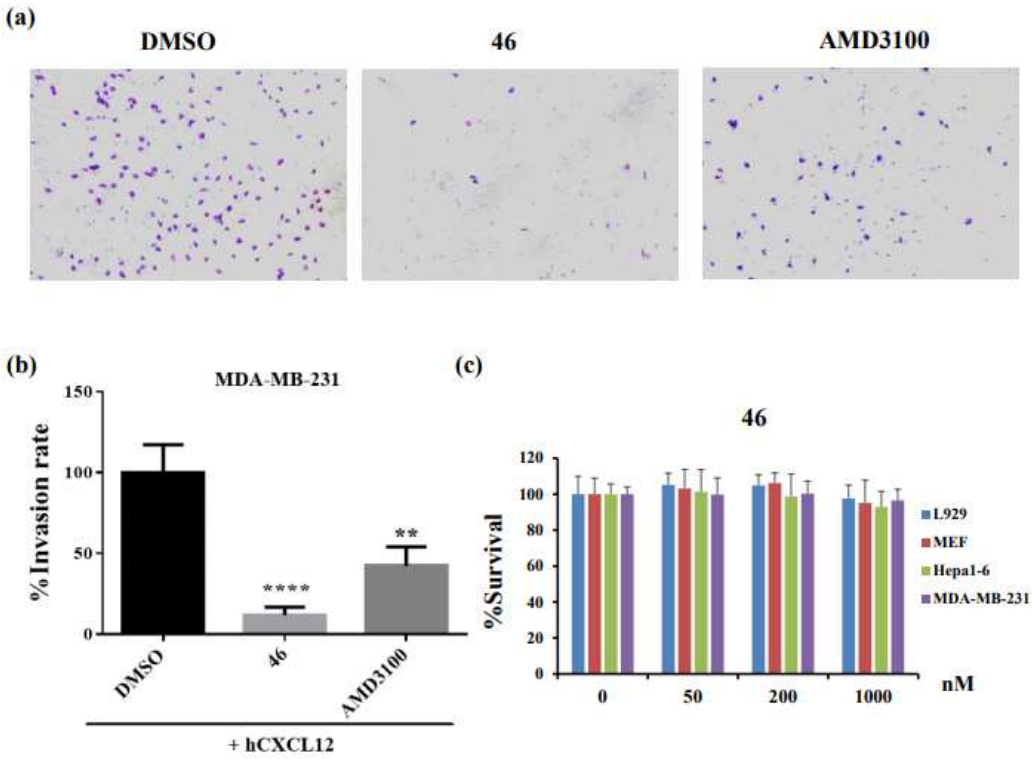


Fig. 4. Effect of compound **46** on matrigel invasion of MDA-MB-231 cells and cytotoxicity assessment of **46** in the cultured human and murine cells. (a) Photo images of matrigel 22 h after invasion experiment. (b) Quantification of transwell analysis of cell invasion experiment. Data represent mean value ± standard deviation. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Magnification: 100x. (c) Effect of compound **46** on proliferation of human and murine cells.

4.3. *Preliminary in vitro safety and metabolic stability evaluation of lead compound **46***

AMD11070 was derived from AMD3100 with reduce molecular weight and cationic charges. As a result, AMD11070 can be dosed orally and has been progressed into clinical testing. However, it was reported that AMD11070 suffered from CYP isozyme inhibition (3A4, 2D6; 60% and 64%, respectively, at 10 μM), possibly due to the benzimidazole functionality [28]. Compound **46** possesses good physicochemical properties (MW 367, $logD_{7.4}$ 1.12, pKa 8.2) and was evaluated by *in vitro* safety tests. As shown in Table 4, compound **46** exhibited minimal inhibition of CYP 1A2, 2C9, 2C19, 2D6, and 3A4 at 10 μM concentration. Moreover, compound **46** exhibited very low inhibition (IC_{50} > 30 μM) of hERG (standard patch clamp), indicating minimal cardiotoxicity liability associated with blockade of this key potassium channel. In addition, as shown in Table 5, compound **46** displayed low plasma protein binding in mouse and rat (33% and 44%, respectively) but high plasma protein binding in human (95%). Although it is common for compounds to have higher plasma protein binding in human than in preclinical species, the variation of plasma protein binding in rodents and in human is large in this case.[35] Since compound **46** is lipophilic ($logD_{7.4}$ 1.12) and basic (pKa 8.2), it likely binds to albumin and more so, to α1-acid glycoprotein.[36] The observed species difference of the plasma protein binding might reflect the different interactions between compound **46** and α1-acid glycoprotein/albumin in rodents and human. More importantly, the higher unbound fractions in rodents may lead to higher clearance compare with human.[37] Similarly, the higher unbound fractions in

rodents may also impose advantage on efficacy in rodent disease models. These factors must be considered for further progression of compound **46**. Lastly, compound **46** demonstrated moderate intrinsic clearance in rat (123 mL/min/kg) and human (66 mL/min/kg) liver microsomes. This is in contrast to the poor metabolic stability exhibited by our former results (Table 1) [28, 29] and represents a major improvement in drug candidate optimization.

Table 4. Preliminary *in vitro* safety evaluation of compound **46**

Cmpd	hERG IC_{50} (μM)	CYP inhibition (%), test concentration (10 μM)					
		1A2	2C9	2C19	2D6	3A4 (midazolam)	3A4 (testosterone)
46	> 30	-6	1	11	-5	7	-4

Table 5. Preliminary *in vitro* DMPK evaluation of compound **46**

Cmpd[a]	PPB (%)			RLM[b]		HLM[c]	
	mouse	rat	human	Cl_{int} (mL/min/kg)	$t_{1/2}$ (min)	Cl_{int} (mL/min/kg)	$t_{1/2}$ (min)
46	33	44	95	123	20.2	66	26.2

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

4.4. *Evaluation of compound **46** in cancer metastasis model in mice*

Cancer cells expressing CXCR4 can migrate to distant CXCL12-rich tissues such as bone marrow, lungs, and lymph nodes. CXCR4 antagonists have the potential to blockade this process and therefore inhibit cancer metastasis, which is a predominant factor for poor patient prognosis in many cancers. We have established a lung metastasis mouse model using red florescence protein labeled Lewis lung carcinoma LL/2 (RFP-LL/2) [38]. Compound **46** was evaluated in this model for its potential as an anti-cancer metastasis agent. As shown in Figure 5a, C57BL/6 mice received tail intravenous injection of RFP-LL/2 and developed lung metastasis. After 18 days, mice were sacrificed and lung tissues were collected. The lung metastasis of LL/2 cells in mice was analyzed by measuring RFP fluorescence intensities with the PerkinElmer IVIS Lumina II. Treatment by compound **46** (70 mg/kg D-tartaric acid salt, equivalent to 30 mg/kg active pharmaceutical ingredient, i.p.) significantly reduced the lung metastasis of LL/2 cells, indicating the potential of compound **46** as an anti-cancer metastasis agent (Figure 5, b, c, and d).

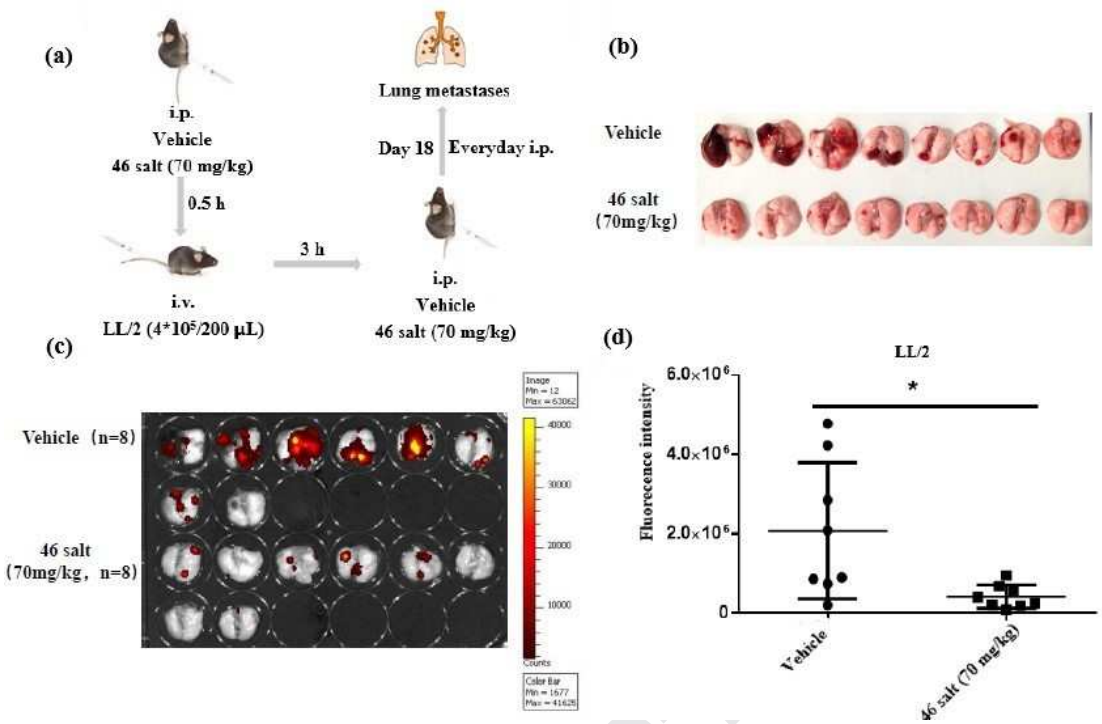


Fig. 5. *In vivo* anti-metastasis evaluation of compound **46** salt. (a) Schematic of tumor metastasis assay. (b) All images of the lungs taken by the camera. (c, d) Tumor metastasis of RFP-labeled LL/2 was calculated by RFP luciferase intensity. Representative pictures and statistical diagram are present. *P < 0.05.

5. Conclusion

Herein we report the design, synthesis and evaluation of novel CXCR4 antagonists based on a pyrrolidine scaffold. The structural exploration/optimization identified compound **46** as a potent CXCR4 antagonist. Compound **46** competitively displaced fluorescent 12G5 antibody with IC_{50} = 79 nM and inhibited CXCL12 induced cytosolic calcium flux (IC_{50} = 0.25 nM). Moreover, in a transwell invasion assay, compound **46** significantly mitigated CXLC12/CXCR4 mediated cell migration. Compound **46** exhibited good physicochemical properties (MW 367, $logD_{7.4}$ 1.12, pKa 8.2) and excellent *in vitro* safety profiles (e.g., hERG patch clamp IC_{50} > 30 μM and minimal CYP isozyme inhibition). Importantly, **46** displayed much improved metabolic stability in human and rat liver microsomes. Lastly, **46** demonstrated marked efficacy in a mouse cancer metastasis model. These results strongly support **46** as a prototypical lead for the development of promising CXCR4 antagonists as clinical candidates.

6. Experimental protocols

6.1. Chemistry

General reaction progress was monitored by analytical thin layer chromatography performed on silica gel HSGF254 pre-coated plates. Organic solutions were dried over anhydrous Na_2SO_4, and the solvents were removed under reduced pressure. Final compounds were purified with silica gel 100-200 mesh for column chromatography. ^{1}H NMR were obtained on 400 MHz (Varian) spectrometer, and ^{13}C

NMR were obtained on 600 MHz (Varian) spectrometer. Chemical shifts were given in ppm using tetramethylsilane as internal standard. Mass spectra were obtained using an Agilent 1100 LC/MSD Trap SL version Mass Spectrometer. HRMS analysis was recorded on an Agilent 6540 UHD Accurate-Mass Q-TOF LC/MS. Values of optical rotation were measured on a Rudolph Automatic Polarimeter A21101. 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.

*6.1.1. 2-(Piperidin-2-yl)pyridine (**15**)*

To a solution of **14** (3.1 g, 20 mmol), tin (9.4 g, 80 mmol) in ethanol (50 mL) was slowly added 6 N HCl aqueous solution (40 mL) and stirred at 100 ^{o}C for 12 h. The reaction mixture was slowly added saturated NaOH aqueous solution to adjust pH 8-9. The water layer was extracted with dichloromethane (100 mL * 3), dried over Na_2SO_4, filtered and concentrated. To a solution of the resulting residue in methanol (40 mL) was added 10% Pd/C (300 mg) and stirred at room temperature under H_2 atmosphere for 16 h. The mixture was filtrated and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/methanol/ammonia = 100/1/1) to give the desired product (200 mg, 6.3%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, *J* = 4.0 Hz, 1H), 7.63 (t, *J* = 7.6 Hz, 1H), 7.65-7.59 (m, 1H), 7.19-7.10 (m, 1H), 4.25 (t, *J* = 7.6 Hz, 1H), 3.25-3.20 (m, 1H), 3.05-2.99 (m, 1H), 2.62-2.32 (m, 2H), 2.27-2.19 (m, 1H), 1.94-1.82 (m, 2H), 1.79-1.70 (m, 1H). MS (ESI/APCI) m/z 163.0 $[M+H]^+$.

*6.1.2. 2-(3,4-Dihydro-2H-pyrrol-5-yl)pyridine (**17**)*

To a solution of 60% NaH (3.2 g, 80 mmol) in dry THF (100 mL) was slowly added 1-vinylpyrrolidin-2-one (4.5 g, 40 mmol) and **16** (6.0 g, 40 mmol) at 0 ^{o}C under N_2 atmosphere. The reaction mixture was heated to 60 ^{o}C and stirred at the same temperature for 2 h. The mixture was cooled to room temperature, 4 N HCl aqueous solution (50 mL) was added and stirred at room temperature overnight. 20% NaOH aqueous solution (50 mL) was added and extracted with dichloromethane (50 mL * 3). The combined organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 100/1) to give the desired product (1.1 g, 19%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, *J* = 4.4 Hz, 1H), 8.11 (d, *J* = 8.0 Hz, 1H), 7.78-7.70 (m, 1H), 7.35-7.28 (m, 1H), 4.15-4.08 (m, 2H), 3.14-3.06 (m, 2H), 2.14-1.94 (m, 2H). MS (ESI/APCI) m/z 147.0 $[M+H]^+$.

*6.1.3. 2-(Pyrrolidin-2-yl)pyridine (**18**)*

To a solution of **17** (290 mg, 2.0 mmol) and acetic acid (2 mL) in methanol (8 mL) was slowly added $NaBH_4$ (190 mg, 5 mmol) at -20 ^{o}C for 3 h. The reaction was quenched with water (20 mL) and washed with ethyl acetate (30 mL). The water layer was adjusted pH to 9 by saturated $NaHCO_3$ aqueous solution and extracted with dichloromethane (20 mL * 4). The combined organic layer was dried over Na_2SO_4, filtered and concentrated to give the desired product (126 mg, 43%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56-8.47 (m, 1H), 7.67-7.59 (m, 1H), 7.40-7.30 (m, 1H), 7.19-7.10 (m, 1H), 4.27 (t, *J* = 7.2 Hz, 1H), 3.28-3.16 (m, 1H), 3.07-2.98 (m, 1H), 2.30-2.15 (m, 1H), 1.95-1.84 (m, 2H), 1.80-1.69 (m, 1H). MS (ESI/APCI) m/z 149.0 $[M+H]^+$.

*6.1.4. Ethyl 4-((diphenylmethylene)amino)-3-methyl-4-(pyridin-2-yl)butanoate (**20**)*

A mixture of **19** (400 mg 3.7 mmol) and benzophenone (670 mg, 3.7 mmol) in toluene (10 mL) was stirred at 120 ^{o}C with Dean-Stark trap overnight. The mixture was cooled to room temperature and

washed with saturated $NaHCO_3$ aqueous solution (10 mL * 2). The organic layer was dried over Na_2SO_4, filtered and concentrated. To a solution of the resulting residue in acetonitrile (15 mL) was added 50% NaOH aqueous solution (0.23 mL, 2.8 M), benzyltriethylammonium chloride (60 mg, 0.30 mmol) and stirred at room temperature for 30 min. Ethyl (*E*)-but-2-enoate (630 mg, 5.5 mmol) was added and the mixture was stirred at room temperature overnight. The mixture was diluted with water (20 mL) and extracted with dichloromethane (20 mL * 3). The combined organic phase was dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 10/1) to give the desired product (1.3 g, 92%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, *J* = 4.0 Hz, 1H), 7.75-7.68 (m, 2H), 7.67-7.58 (m, 1H), 7.45-7.32 (m, 7H), 7.16-7.10 (m, 1H), 7.03-6.90 (m, 2H), 4.53-4.38 (m, 1H), 4.08-3.98 (m, 2H), 2.89-2.70 (m, 1H), 2.58-2.05 (m, 2H), 1.25-1.11 (m, 3H), 1.00-0.80 (m, 3H). MS (ESI/APCI) m/z 386.9 $[M+H]^+$.

*6.1.5. 4-Methyl-5-(pyridin-2-yl)pyrrolidin-2-one (**21**)*

To a solution of **20** (1.3 g, 3.3 mmol) in acetonitrile (15 mL) was added dropwise conc. HCl solution (3 mL) and stirred at room temperature for 2 h. The mixture was diluted with water (20 mL) and extracted with dichloromethane (20 mL * 3). The combined organic phase was dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 100/1) to give the desired product (360 mg, 62%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64-8.54 (m, 1H), 7.77-7.65 (m, 1H), 7.37-7.29 (m, 0.7H), 7.26-7.20 (m, 1.3H), 6.14-5.93 (m, 1H), 4.88 (d, *J* = 7.6 Hz, 0.3H), 4.39 (d, *J* = 6.0 Hz, 0.7H), 3.04-2.90 (m, 0.3H), 2.69-2.41 (m, 1.7 H), 2.25-2.06 (m, 1H), 1.27 (d, *J* = 6.8 Hz, 2.1H), 0.67 (d, *J* = 7.2 Hz, 0.9 H). MS (ESI/APCI) m/z 177.1 $[M+H]^+$.

*6.1.6. 2-(3-Methylpyrrolidin-2-yl)pyridine (**22**)*

To a solution of **21** (200 mg, 1.1 mmol) in THF (5 mL) was added $LiAlH_4$ (170 mg, 4.5 mmol) slowly and stirred at 50 ^{o}C overnight. The reaction mixture was cooled to room temperature. Water (0.2 mL), 25% NaOH solution (0.2 mL) and water (0.6 mL) were added in sequence to quench the reaction. The mixture was filtrated, concentrated and purified by silica gel column chromatography (dichloromethane/methanol/ammonia = 100/1/1) to give the desired product (89 mg, 50%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61-8.48 (m, 1H), 7.70-7.60 (m, 1H), 7.34-7.28 (m, 1H), 7.21-7.14 (m, 1H), 4.49 (d, *J* = 7.2 Hz, 0.3H), 3.75 (d, *J* = 8.0 Hz, 0.7H), 3.32-3.10 (m, 2H), 2.63-2.53 (m, 1H), 2.17-2.03 (m, 2H), 1.64-1.55 (m, 1H), 1.08 (d, *J* = 6.0 Hz, 2.1H), 0.58 (d, *J* = 6.8 Hz, 0.9H). MS (ESI/APCI) m/z 163.0 $[M+H]^+$.

*6.1.7. General procedure for the synthesis of **23-25***

A mixture of **15**/ **18**/ **22** (1.0 mmol, 1.0 eq), 4-chloro-6-(chloromethyl)-2-methylpyrimidine (1.1 mmol, 1.1 eq**)**, KI (0.10 mmol, 0.10 eq), and DIPEA (2.5 mmol, 2.5 eq) in acetonitrile (5 mL) was stirred at room temperature overnight. The mixture was concentrated and purified by basic Al_2O_3 column chromatography (petroleum ether/ethyl acetate = 1/1) to give the desired product.

*6.1.7.1. 4-Chloro-2-methyl-6-((2-(pyridin-2-yl)piperidin-1-yl)methyl)pyrimidine (**23**)*

Compound **23** was obtained as a yellow oil (yield 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, *J* = 4.4 Hz, 1H), 7.65-7.59 (m, 1H), 7.52-7.37 (m, 2H), 7.19-7.09 (m, 1H), 3.62 (d, *J* = 16.4 Hz, 1H), 3.52-3.42 (m, 1H), 3.20 (d, *J* = 16.4 Hz, 1H), 3.00-2.89 (m, 1H), 2.61 (s, 3H), 2.27-2.13 (m, 1H), 1.95-1.81 (m, 2H), 1.73-1.62 (m, 3H), 1.52-1.44 (m, 1H). MS (ESI/APCI) m/z 302.8 $[M+H]^+$.

*6.1.7.2. 4-Chloro-2-methyl-6-((2-(pyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidine (**24**)*

Compound **24** was obtained as a colorless oil (yield 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, *J* = 4.4 Hz, 1H), 7.69-7.59 (m, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.35 (s, 1H), 7.19-7.11 (m, 1H), 3.83 (d, *J* =

16 Hz, 1H), 3.79-3.70 (m, 1H), 3.52 (d, *J* = 16.0 Hz, 1H), 3.34-3.23 (m, 1H), 2.64 (s, 3H), 2.50-2.30 (m, 2H), 2.10-1.81 (m, 3H). MS (ESI/APCI) m/z 288.8 $[M+H]^+$.

*6.1.7.3. 4-Chloro-2-methyl-6-((3-methyl-2-(pyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidine (**25**)*

Compound **25** was obtained as a colorless oil (yield 13%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62-8.48 (m, 1H), 7.70-7.59 (m, 1H), 7.51-7.42 (m, 1H), 7.40-7.30 (m, 1H), 7.22-7.09 (m, 1H), 4.15-3.92 (m, 0.3H), 3.93-3.67 (m, 1H), 3.61-3.40 (m, 1H), 3.35-3.20 (m, 1.7H), 2.68-2.57 (m, 3H), 2.54-2.40 (m, 1H), 2.28-2.15 (m, 2H), 1.69-1.47 (m, 1H), 1.04 (d, *J* = 6.0 Hz, 2.5H), 0.58 (d, *J* = 7.2 Hz, 0.5H). MS (ESI/APCI) m/z 302.9 $[M+H]^+$.

*6.1.8. General procedure for the synthesis of **26-28***

A mixture of **23-25** (0.20 mmol, 1.0 eq), *N*-methylpiperazine (1.0 mmol, 5.0 eq) and TEA (2.0 mmol, 10 eq) in ethanol (5 mL) was stirred at 85 °C for 3 h. The mixture was concentrated and purified by basic Al_2O_3 column chromatography (dichloromethane/methanol = 100/1) to give the desired product.

*6.1.8.1. 2-Methyl-4-(4-methylpiperazin-1-yl)-6-((2-(pyridin-2-yl)piperidin-1-yl)methyl)pyrimidine (**26**)*

Compound **26** was obtained as a colorless oil (yield 38%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48-8.41 (m, 1H), 7.60-7.52 (m, 1H), 7.39 (d, *J* = 8.0 Hz, 1H), 7.09-7.02 (m, 1H), 6.55 (s, 1H), 3.67-3.58 (m, 4H), 3.48 (d, *J* = 16.0 Hz, 1H), 3.42-3.33 (m, 1H), 3.09-2.95 (m, 2H), 2.47-2.41 (m, 4H), 2.37 (s, 3H), 2.30 (s, 3H), 2.15-2.05 (m, 1H), 1.88-1.72 (m, 2H), 1.68-1.56 (m, 3H), 1.47-1.33 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.9, 166.6, 164.1, 162.6, 149.0, 136.6, 122.0, 121.5, 97.1, 70.2, 61.0, 54.7, 54.0, 46.2, 43.7, 34.9, 26.0, 25.8, 24.6. HRMS (ESI): calcd for $C_{21}H_{30}N_6$ $[M+H]^+$ 367.2605, found 367.2606. Purity: 99.1%.

*6.1.8.2. 2-Methyl-4-(4-methylpiperazin-1-yl)-6-((2-(pyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidine (**27**)*

Compound **27** was obtained as a colorless oil (yield 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, *J* = 4.0 Hz, 1H), 7.63-7.55 (m, 1H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.14-7.06 (m, 1H), 6.44 (s, 1H), 3.74-3.66 (m, 2H), 3.64-3.58 (m, 4H), 3.38 (d, *J* = 15.2 Hz, 1H), 3.34-3.27 (m, 1H), 2.48-2.41 (m, 8H), 2.34-2.24 (m, 4H), 2.03-1.95 (m, 1H), 1.93-1.80 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.7, 166.6, 163.5, 162.6, 149.2, 136.6, 122.0, 121.5, 97.9, 71.0, 60.3, 54.8, 54.6, 46.2, 43.7, 33.5, 26.1, 23.2. HRMS (ESI): calcd for $C_{20}H_{28}N_6$ $[M+H]^+$ 353.2448, found 353.2455. Purity: 99.0%.

6.1.8.3.

*2-Methyl-4-((3-methyl-2-(pyridin-2-yl)pyrrolidin-1-yl)methyl)-6-(4-methylpiperazin-1-yl)pyrimidine (**28**)*

Compound **28** was obtained as a colorless oil (yield 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60-8.48 (m, 1H), 7.65-7.54 (m, 1H), 7.47-7.38 (m, 1H), 7.18-7.08 (m, 1H), 6.48-6.38 (m, 1H), 3.93 (d, *J* = 8.8 Hz, 0.1H), 3.71-3.62 (m, 5H), 3.38-3.26 (m, 2H), 3.21 (d, *J* = 7.6 Hz, 0.9H), 2.51-2.38 (m, 8H), 2.34 (s, 3H), 2.27-2.15 (m, 2H), 1.58-1.47 (m, 1H), 1.04 (d, *J* = 5.6 Hz, 2.7H), 0.57 (d, *J* = 7.2 Hz, 0.3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.7, 166.6, 162.6, 162.5, 149.2, 136.5, 122.2, 122.0, 97.9, 79.1, 60.6, 54.8, 53.3, 46.3, 43.8, 41.9, 31.9, 26.1, 18.4. HRMS (ESI): calcd for $C_{21}H_{30}N_6$ $[M+H]^+$ 367.2605, found 367.2621. Purity: 99.2%.

*6.1.9. 3-(1,3-Dioxolan-2-yl)-1-(pyridin-2-yl)propan-1-one (**30**)*

To a solution of Mg (1.1 g, 44 mmol) and I_2 (20 mg) in dry THF (10 mL) at room temperature was added the THF solution (2 mL) of 2-(2-bromoethyl)-1,3-dioxolane (696 mg, 4 mmol) under nitrogen protection. The mixture was stirred at 40-50 °C until the iodine color disappeared. The THF solution (18 mL) of 2-(2-bromoethyl)-1,3-dioxolane solution (6.5 g, 36 mmol) was then added dropwise to the turnings at room temperature to keep the inner temperature below 30 °C. The reaction was stirred at room temperature for an additional 1.5 h. This mixture was added dropwise to the solution of **29** (2.1 g,

20 mmol) in dry THF (50 mL) at 0 ^{o}C and stirred at the same temperature for 2h. The saturated ammonium chloride solution (20 mL) was added, extracted with ethyl acetate (30 mL * 3). The combined organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 10/1) to give the desired product (2.5 g, 61%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.12-7.95 (m, 1H), 7.87-7.72 (m, 1H), 7.52-7.40 (m, 1H), 5.02 (s, 1H), 4.00-3.92 (m, 2H), 3.88-3.82 (m, 2H), 3.36 (t, *J* = 7.2 Hz, 2H), 2.20-2.10 (m, 2H). MS (ESI/APCI) m/z 207.9 $[M+H]^+$.

*6.1.10. 4-Oxo-4-(pyridin-2-yl)butanal (**31**)*

To a solution of **30** (1.3 g, 6.0 mmol) in acetone (20 mL) was added 3 N HCl aqueous solution (10 mL, 30 mmol) and stirred at room temperature overnight. The reaction mixture was added saturated Na_2CO_3 aqueous solution to adjust pH to 7 and extracted with dichloromethane (20 mL * 3). The combined organic phase was dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 10/1) to give the desired product (800 mg, 82%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (s, 1H), 8.68 (d, *J* = 4.0 Hz, 1H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.89-7.75 (m, 1H), 7.56-7.43 (m, 1H), 3.57 (t, *J* = 6.4 Hz, 2H), 2.91 (t, *J* = 6.4 Hz, 2H). MS (ESI/APCI) m/z 164.0 $[M+H]^+$.

*6.1.11. 2-((S)-1-((R)-1-(4-Methoxyphenyl)ethyl)pyrrolidin-2-yl)pyridine (**32**)*

To a solution of **31** (490 mg, 3.0 mmol) and acetic acid (18 mg, 0.30 mmol) in dichloromethane (15 mL) was slowly added $NaBH(OAc)_3$ (1.9 g, 9.0 mmol) at -70 ^{o}C and stirred at the same temperature for 30 min. (R)-1-(4-methoxyphenyl)ethan-1-amine (500 mg, 3.3 mmol) was added and stirred at room temperature overnight. The mixture was quenched with water (5 mL), concentrated and purified by basic Al_2O_3 column chromatography (petroleum ether/ethyl acetate = 1/1) to give the desired product (410 mg, 48%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49-8.35 (m, 1H), 7.63-7.48 (m, 1H), 7.44-7.33 (m, 1H), 7.20-7.10 (m, 2H), 7.10-7.00 (m, 1H), 6.79-6.65 (m, 2H), 4.05-3.92 (m, 1H), 3.80-3.71 (m, 4H), 3.11 (s, 1H), 2.63 (s, 1H), 2.24 (s, 1H), 1.99-1.85 (m, 1H), 1.76 (s, 2H), 1.35 (d, *J* = 4.8 Hz, 3H). MS (ESI/APCI) m/z 282.9 $[M+H]^+$. $[\alpha]_D^{25}$ = -11 (c = 0.2, MeOH).

*6.1.12. (S)-2-(Syrrolidin-2-yl)pyridine (**33**)*

A mixture of **33** (410 mg, 1.5 mmol) in TFA (5 mL) was stirred at 50 ^{o}C for 12 h. The mixture was concentrated, dissolved in 1N HCl aqueous solution (10 mL) and then washed with dichloromethane (10 mL * 3). The water layer was added saturated Na_2CO_3 aqueous solution to adjust pH to 9 and extracted with dichloromethane (20 mL * 3). The combined organic phase was dried over Na_2SO_4, filtered and concentrated to give the desired product (110 mg, 50%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, *J* = 4.4 Hz, 1H), 7.73-7.62 (m, 1H), 7.33 (d, *J* = 7.6 Hz, 1H), 7.23-7.16 (m, 1H), 4.67-4.52 (m, 1H), 3.39-3.24 (m, 2H), 2.45-2.32 (m, 1H), 2.06-1.82 (m, 3H). MS (ESI/APCI) m/z 149.0 $[M+H]^+$.

*6.1.13. (S)-4-Chloro-2-methyl-6-((2-(pyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidine (**34**)*

A mixture of **33** (110 mg, 0.74 mmol), 4-chloro-6-(chloromethyl)-2-methylpyrimidine (150 mg, 0.81mmol**)**, KI (12 mg, 0.074 mmol), and DIPEA (240 mg, 1.9 mmol) in acetonitrile (5 mL) was stirred at room temperature overnight. The mixture was concentrated and purified by basic Al_2O_3 column chromatography (petroleum ether/ethyl acetate = 1/1) to give the desired product (68 mg, 32%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58-8.47 (m, 1H), 7.68-7.60 (m, 1H), 7.47 (d, *J* = 7.6 Hz, 1H), 7.38-7.32 (m, 1H), 7.17-7.09 (m, 1H), 3.82 (d, *J* = 16.0 Hz, 1H), 3.78-3.70 (m, 1H), 3.51 (d, *J* = 15.6 Hz, 1H), 3.34-3.20 (m, 1H), 2.64 (s, 3H), 2.49-2.47 (m, 2H), 2.11-1.80 (m, 3H). MS (ESI/APCI) m/z 288.8 $[M+H]^+$. $[\alpha]_D^{25}$ = -2 (c = 0.2, MeOH).

*6.1.14. (S)-2-Methyl-4-(4-methylpiperazin-1-yl)-6-((2-(pyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidine (**35**)*

A mixture of **34** (68 mg, 0.24 mmol), *N*-methylpiperazine (120 mg, 1.2 mmol) and TEA (240 mg, 2.4 mmol) in ethanol (5 mL) was stirred at 85 ^{o}C for 3 h. The mixture was concentrated and purified by basic Al_2O_3 column chromatography (dichloromethane/methanol = 100/1) to give the desired product (70 mg, 83 %) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55-8.41 (m, 1H), 7.62-7.51 (m, 1H), 7.41 (d, *J* = 7.6 Hz, 1H), 7.13-6.99 (m, 1H), 6.41 (s, 1H), 3.71-3.55 (m, 6H), 3.38-3.25 (m, 2H), 2.45-2.35 (m, 8H), 2.33-2.20 (m, 4H), 2.00-1.75 (m, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.6, 166.5, 163.5, 162.6, 149.1, 136.5, 121.9, 121.4, 97.8, 71.0, 60.3, 54.7, 54.6, 46.2, 43.6, 33.4, 26.0, 23.1. HRMS (ESI): calcd for $C_{20}H_{28}N_6$ $[M+H]^+$ 353.2448, found 353.2450. Purity: 99.2%. $[\alpha]_D^{25}$ = +6 (c = 0.2, MeOH).

*6.1.15. 2-((R)-1-((S)-1-(4-Methoxyphenyl)ethyl)pyrrolidin-2-yl)pyridine (**36**)*

To a solution of **31** (330 mg, 2.0 mmol) and acetic acid (18 mg, 0.30 mmol) in dichloromethane (15 mL) was added $NaBH(OAc)_3$ (1.3 g, 6 mmol) slowly at -70 ^{o}C and stirred at the same temperature for 30 min. (S)-1-(4-methoxyphenyl)ethan-1-amine (330 mg, 2.2 mmol) was added and stirred at room temperature overnight. The mixture was quenched with water (5 mL), concentrated and purified by basic Al_2O_3 column chromatography (petroleum ether/ethyl acetate = 1/1) to give the desired product (400 mg, 71%) as a colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40-8.33 (m, 1H), 7.73-7.62 (m, 1H), 7.47 (d, *J* = 7.6 Hz, 1H), 7.21-7.06 (m, 3H), 6.80-6.68 (m, 2H), 3.97-3.86 (m, 1H), 3.68 (s, 3H), 3.66-3.60 (m, 1H), 2.93-2.76 (m, 1H), 2.65-2.51 (m, 1H), 2.20-2.03 (m, 1H), 1.80-1.55 (m, 3H), 1.22 (d, *J* = 6.0 Hz, 3H). MS (ESI/APCI) m/z 282.9 $[M+H]^+$. $[\alpha]_D^{25}$ = +11 (c = 0.2, MeOH).

*6.1.16. (R)-2-(Pyrrolidin-2-yl)pyridine (**37**)*

A mixture of **36** (400 mg, 1.5 mmol) in TFA (5 mL) was stirred at 50 ^{o}C for 12 h. The mixture was concentrated, dissolved in 1 N HCl (10 mL) and washed with dichloromethane (10 mL * 3). The water layer was added saturated Na_2CO_3 aqueous solution to adjust pH to 9 and extracted with dichloromethane (20 mL * 3). The combined organic phase was dried over Na_2SO_4, filtered and concentrated to give the desired product (100 mg, 45%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57-8.47 (m, 1H), 7.70-7.54 (m, 1H), 7.40-7.30 (m, 1H), 7.19-7.08 (m, 1H), 4.30-4.17 (m, 1H), 3.27-3.15 (m, 1H), 3.08-2.92 (m, 1H), 2.30-2.15 (m, 1H), 1.90-1.70 (m, 3H). MS (ESI/APCI) m/z 149.0 $[M+H]^+$.

*6.1.17. (R)-4-Chloro-2-methyl-6-((2-(pyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidine (**38**)*

A mixture of **37** (100 mg, 0.67 mmol), 4-chloro-6-(chloromethyl)-2-methylpyrimidine (130 mg, 0.74 mmol**)**, KI (11 mg, 0.067 mmol), and DIPEA (220 mg, 1.7 mmol) in acetonitrile (5 mL) was stirred at room temperature overnight. The mixture was concentrated and purified by basic Al_2O_3 column chromatography (petroleum ether/ethyl acetate = 1/1) to give the desired product (60 mg, 31%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, *J* = 4.0 Hz, 1H), 7.68-7.60 (m, 1H), 7.51-7.45 (m, 1H), 7.35 (s, 1H), 7.18-7.09 (m, 1H), 3.82 (d, *J* = 16.0 Hz, 1H), 3.79-3.70 (m, 1H), 3.51 (d, *J* = 15.6 Hz, 1H), 3.34-3.23 (m, 1H), 2.64 (s, 3H), 2.48-2.30 (m, 2H), 2.08-1.81 (m, 3H). MS (ESI/APCI) m/z 288.8 $[M+H]^+$. $[\alpha]_D^{25}$ = +2 (c = 0.2, MeOH).

*6.1.18. (R)-2-Methyl-4-(4-methylpiperazin-1-yl)-6-((2-(pyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidine (**39**)*

A mixture of **38** (60 mg, 0.21 mmol), *N*-methylpiperazine (104 mg, 1.0 mmol) and TEA (210 mg, 2.1 mmol) in ethanol (5 mL) was stirred at 85 ^{o}C for 3 h. The mixture was concentrated and purified by basic Al_2O_3 column chromatography (dichloromethane/methanol = 100/1) to give the desired product

(50 mg, 68%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, *J* = 4.0 Hz, 1H), 7.65-7.54 (m, 1H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.15-7.08 (m, 1H), 6.45 (s, 1H), 3.72-3.68 (m, 2H), 3.65-3.58 (m, 4H), 3.40-3.31 (m, 2H), 2.50-2.40 (m, 8H), 2.36-2.26 (m, 4H), 2.05-1.83 (m, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.7, 166.6, 163.5, 162.6, 149.2, 136.6, 122.0, 121.5, 97.9, 71.0, 60.3, 54.8, 54.6, 46.2, 43.7, 33.5, 26.1, 23.2. HRMS (ESI): calcd for $C_{20}H_{28}N_6$ $[M+H]^+$ 353.2448, found 353.2450. Purity: 96.2%. $[\alpha]_D^{25}$ = -6 (c = 0.2, MeOH).

*6.1.19. General procedure for the synthesis of **41a-d***

To a solution of Mg (44 mmol, 2.2 eq) and I_2 (20 mg) in dry THF (10 mL) at room temperature was added the THF solution (2 mL) of 2-(2-bromoethyl)-1,3-dioxolane (4.0 mmol, 0.20 eq) under nitrogen protection. The mixture was stirred at 40-50 ^{o}C until the iodine color disappeared. The THF solution (18 mL) of 2-(2-bromoethyl)-1,3-dioxolane (36 mmol, 1.8 eq) was then added dropwise to the turnings at room temperature to keep the inner temperature below 30 ^{o}C. The reaction was stirred at room temperature for an additional 1.5 h. This mixture was added dropwise to the solution of **40a-d** (20 mmol, 1.0 eq) in dry THF (50 mL) at 0 ^{o}C and stirred at the same temperature for 2 h. The saturated ammonium chloride solution (20 mL) was added, extracted with ethyl acetate (30 mL * 3). The combined organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 10/1) to give the desired product.

*6.1.19.1. 3-(1,3-Dioxolan-2-yl)-1-(3-methylpyridin-2-yl)propan-1-one (**41a**)*

Compound **41a** was obtained as a colorless oil (yield 49%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55-8.45 (m, 1H), 7.76 (d, *J* = 7.6 Hz, 1H), 7.54-7.45 (m, 1H), 4.92-4.80 (m, 1H), 3.87-3.80 (m, 2H), 3.77-3.71 (m, 2H), 3.17 (t, *J* = 7.2 Hz, 2H), 2.46 (s, 3H), 1.98-1.85 (m, 2H). MS (ESI/APCI) m/z 221.9 $[M+H]^+$.

*6.1.19.2. 3-(1,3-Dioxolan-2-yl)-1-(4-methylpyridin-2-yl)propan-1-one (**41b**)*

Compound **41b** was obtained as a colorless oil (yield 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, *J* = 4.8 Hz, 1H), 7.85 (s, 1H), 7.32-7.26 (m, 1H),5.02 (t, *J* = 4.0 Hz, 1H), 4.00-3.92 (m, 2H), 3.88-3.80 (m, 2H), 3.34 (t, *J* = 7.6 Hz, 2H), 2.41 (s, 3H), 2.18-2.10 (m, 2H). MS (ESI/APCI) m/z 221.9 $[M+H]^+$.

*6.1.19.3. 3-(1,3-Dioxolan-2-yl)-1-(5-methylpyridin-2-yl)propan-1-one (**41c**)*

Compound **41c** was obtained as a colorless oil (yield 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H), 7.92 (d, *J* = 7.6 Hz, 1H), 7.60 (d, *J* = 7.6 Hz, 1H), 5.05-4.98 (m, 1H), 3.97-3.92 (m, 2H), 3.86-3.82 (m, 2H), 3.32 (t, *J* = 7.2 Hz, 2H), 2.39 (s, 3H), 2.20-2.10 (m, 2H). MS (ESI/APCI) m/z 221.9 $[M+H]^+$.

*6.1.19.4. 3-(1,3-Dioxolan-2-yl)-1-(6-methylpyridin-2-yl)propan-1-one (**41d**)*

Compound **41d** was obtained as a colorless oil (yield 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, *J* = 7.6 Hz, 1H), 7.72-7.65 (m, 1H), 7.30 (d, *J* = 7.6 Hz, 1H), 5.03 (t, *J* = 4.0 Hz, 1H), 3.99-3.93 (m, 2H), 3.87-3.84 (m, 2H), 3.36 (t, *J* = 7.6 Hz, 2H), 2.60 (s, 3H), 2.19-2.06 (m, 2H). MS (ESI/APCI) m/z 221.9 $[M+H]^+$.

*6.1.20. General procedure for the synthesis of **42a-d***

To a solution of **41a-d** (6.0 mmol, 1.0 eq) in acetone (20 mL) was added 3 N HCl aqueous solution (30 mmol, 5.0 eq) and stirred at room temperature overnight. The reaction mixture was added saturated Na_2CO_3 aqueous solution to adjust pH to 7 and extracted with dichloromethane (20 mL * 3). The combined organic phase was dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 10/1) to give the desired product.

*6.1.20.1. 4-(3-Methylpyridin-2-yl)-4-oxobutanal (**42a**)*

Compound **42a** was obtained as a colorless oil (yield 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (s, 1H), 8.60-8.38 (m, 1H), 7.65-7.46 (m, 1H), 7.39-7.26 (m, 1H), 3.61-3.35 (m, 2H), 2.95-2.72 (m, 2H),

2.56 (s, 3H). MS (ESI/APCI) m/z 177.9 $[M+H]^+$.

*6.1.20.2. 4-(4-Methylpyridin-2-yl)-4-oxobutanal (**42b**)*

Compound **42b** was obtained as a colorless oil (yield 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.88 (s, 1H), 8.53 (d, *J* = 4.8 Hz, 1H), 7.85 (s, 1H), 7.29 (d, *J* = 4.4 Hz, 1H), 3.55 (t, *J* = 6.4 Hz, 2H), 2.90 (t, *J* = 6.4 Hz, 2H), 2.42 (s, 3H). MS (ESI/APCI) m/z 177.9 $[M+H]^+$.

*6.1.20.3. 4-(5-Methylpyridin-2-yl)-4-oxobutanal (**42c**)*

Compound **42c** was obtained as a colorless oil (yield 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.88 (s, 1H), 8.48 (s, 1H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 3.54 (t, *J* = 7.2 Hz, 2H), 2.89 (t, *J* = 6.4 Hz, 2H), 2.41 (s, 3H). MS (ESI/APCI) m/z 177.9 $[M+H]^+$.

*6.1.20.4. 4-(6-Methylpyridin-2-yl)-4-oxobutanal (**42d**)*

Compound **42d** was obtained as a colorless oil (yield 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.90 (s, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.73-7.67 (m, 1H), 7.33 (d, *J* = 7.6 Hz, 1H), 3.58 (t, *J* = 6.4 Hz, 2H), 2.90 (t, *J* = 6.4 Hz, 2H), 2.61 (s, 3H). MS (ESI/APCI) m/z 178.0 $[M+H]^+$.

*6.1.21. General procedure for the synthesis of **43a-d***

To a solution of **42a-d** (2.0 mmol, 1.0 eq) and acetic acid (0.20 mmol, 0.10 eq) in dichloromethane (15 mL) was slowly added $NaBH(OAc)_3$ (6.0 mmol, 3.0 eq) at -70 ^{o}C and stirred at the same temperature for 30 min. (R)-1-(4-methoxyphenyl)ethan-1-amine (2.2 mmol, 1.1 eq) was added and stirred at room temperature overnight. The mixture was quenched with water (5 mL), concentrated and purified by basic Al_2O_3 column chromatography (petroleum ether/ethyl acetate = 1/1) to give the desired product.

*6.1.21.1. 2-((S)-1-((R)-1-(4-Methoxyphenyl)ethyl)pyrrolidin-2-yl)-3-methylpyridine (**43a**)*

Compound **43a** was obtained as a colorless oil (yield 25%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43-8.33 (m, 1H), 7.15-7.06 (m, 1H), 7.05-6.98 (m, 2H), 6.90-6.80 (m, 1H), 6.60-6.47 (m, 2H), 3.99-3.87 (m, 1H), 3.69 (s, 3H), 3.59-3.48 (m, 1H), 3.42-3.29 (m, 1H), 2.60-2.47 (m, 1H), 2.18-2.02 (m, 5H), 1.86-1.72 (m, 2H), 1.31 (d, *J* = 6.8 Hz, 3H). MS (ESI/APCI) m/z 296.9 $[M+H]^+$. $[\alpha]_D^{25}$ = -6 (c = 0.2, MeOH).

*6.1.21.2. 2-((S)-1-((R)-1-(4-Methoxyphenyl)ethyl)pyrrolidin-2-yl)-4-methylpyridine (**43b**)*

Compound **43b** was obtained as a colorless oil (yield 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, *J* = 4.4 Hz, 1H), 7.13 (d, *J* = 7.6 Hz, 2H), 7.05 (s, 1H), 6.82 (d, *J* = 4.4 Hz, 1H), 6.67 (d, *J* = 8.0 Hz, 2H), 3.89-3.75 (m, 1H), 3.74 (s, 3H), 3.70-3.61 (m, 1H), 3.18-3.04 (m, 1H), 2.66- 2.52 (m, 1H), 2.28-2.13 (m, 4H), 1.98-1.84 (m, 1H), 1.87-1.75 (m, 2H), 1.33 (d, *J* = 6.4 Hz, 3H). MS (ESI/APCI) m/z 296.9 $[M+H]^+$. $[\alpha]_D^{25}$ = -16 (c = 0.2, MeOH).

*6.1.21.3. 2-((S)-1-((R)-1-(4-Methoxyphenyl)ethyl)pyrrolidin-2-yl)-5-methylpyridine (**43c**)*

Compound **43c** was obtained as a colorless oil (yield 21%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.35 (d, *J* = 7.2 Hz, 1H), 7.30-7.26 (m, 1H), 7.16 (d, *J* = 8.4 Hz, 2H), 6.71 (d, *J* = 8.4 Hz, 2H), 3.93-3.86 (m, 1H), 3.77-3.68 (m, 4H), 3.09-2.92 (m, 1H), 2.67-2.52 (m, 1H),2.26 (s, 3H), 2.22-2.11 (m, 1H), 1.94-1.82 (m, 1H), 1.81-1.68 (m, 2H), 1.31 (d, *J* = 6.8 Hz, 3H). MS (ESI/APCI) m/z 297.0 $[M+H]^+$. $[\alpha]_D^{25}$ = -15 (c = 0.2, MeOH).

*6.1.21.4. 2-((S)-1-((R)-1-(4-Methoxyphenyl)ethyl)pyrrolidin-2-yl)-6-methylpyridine (**43d**)*

Compound **43d** was obtained as a colorless oil (yield 27%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51-7.42 (m, 1H), 7.32 (d, *J* = 7.6 Hz, 1H), 7.18 (d, *J* = 8.0 Hz, 2H), 6.89 (d, *J* = 7.2 Hz, 1H), 6.72 (d, *J* = 8.4 Hz, 2H), 4.00-3.85 (m, 1H), 3.80-3.70 (m, 4H), 3.09-2.95 (m, 1H), 2.69-2.56 (m, 1H), 2.46 (s, 3H), 2.30-2.15 (m, 1H), 1.96-1.70 (m, 3H), 1.33 (d, *J* = 6.4 Hz, 3H). MS (ESI/APCI) m/z 296.9 $[M+H]^+$. $[\alpha]_D^{25}$ = -5 (c = 0.2, MeOH).

*6.1.22. General procedure for the synthesis of **44a-d***

A mixture of **43a-d** (1.5 mmol) in TFA (5 mL) was stirred at 50 °C for 12 h. The mixture was concentrated, dissolved in 1N HCl aqueous solution (10 mL) and washed with dichloromethane (10 mL * 3). The water layer was added saturated Na_2CO_3 aqueous solution to adjust pH to 9 and extracted with dichloromethane (20 mL * 3). The combined organic phase was dried over Na_2SO_4, filtered and concentrated to give the desired product.

*6.1.22.1. (S)-3-Methyl-2-(pyrrolidin-2-yl)pyridine (**44a**)*

Compound **44a** was obtained as a colorless oil (yield 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42-8.30 (m, 1H), 7.48-7.36 (m, 1H), 7.13-7.00 (m, 1H), 4.46-4.27 (m, 1H), 3.40-3.24 (m, 1H), 3.03-2.88 (m, 1H), 2.34 (s, 3H), 2.27-2.21 (m, 1H), 1.97-1.77 (m, 2H), 1.62-1.46 (m, 1H). MS (ESI/APCI) m/z 163.0 $[M+H]^+$. $[\alpha]_D^{25}$ = -25 (c = 0.2, MeOH).

*6.1.22.2. (S)-4-Methyl-2-(pyrrolidin-2-yl)pyridine (**44b**)*

Compound **44b** was obtained as a colorless oil (yield 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40-8.31 (m, 1H), 7.15 (s, 1H), 7.07-6.95 (m, 1H), 4.57-4.46 (m, 1H), 3.32-3.19 (m, 2H), 2.40-3.30 (m, 4H), 2.02-1.77 (m, 3H). MS (ESI/APCI) m/z 163.0 $[M+H]^+$.

*6.1.22.3. (S)-5-Methyl-2-(pyrrolidin-2-yl)pyridine (**44c**)*

Compound **44c** was obtained as a colorless oil (yield 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.44 (d, *J* = 7.6 Hz, 1H), 7.22 (d, *J* = 7.6 Hz, 1H), 4.27-4.11 (m, 1H), 3.28-3.15 (m, 1H), 3.06-2.93 (m, 1H), 2.30 (s, 3H), 2.24-2.16 (m, 1H), 1.93-1.79 (m, 2H), 1.79-1.65 (m, 1H). MS (ESI/APCI) m/z 163.0 $[M+H]^+$.

*6.1.22.4. (S)-6-Methyl-2-(pyrrolidin-2-yl)pyridine (**44d**)*

Compound **44d** was obtained as a colorless oil (yield 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53-7.45 (t, *J* = 7.6 Hz, 1H), 7.11 (d, *J* = 7.6 Hz, 1H), 6.97 (d, *J* = 7.6 Hz, 1H), 4.20 (t, *J* = 7.6 Hz, 1H), 3.23-3.13 (m, 1H), 3.01-2.97 (m, 1H), 2.50 (s, 3H), 2.25-2.16 (m, 1H), 1.91-1.79 (m, 2H), 1.75-1.66 (m, 1H). MS (ESI/APCI) m/z 163.1 $[M+H]^+$.

*6.1.23. General procedure for the synthesis of **45a-d***

A mixture of **44a-d** (0.67 mmol, 1.0 eq), 4-chloro-6-(chloromethyl)-2-methylpyrimidine (0.74 mmol, 1.1 eq**)**, KI (0.067 mmol, 0.1 eq), and DIPEA (1.7 mmol, 2.5 eq) in acetonitrile (5 mL) was stirred at room temperature overnight. The mixture was concentrated and purified by basic Al_2O_3 column chromatography (petroleum ether/ethyl acetate = 1/1) to give the desired product.

*6.1.23.1. (S)-4-Chloro-2-methyl-6-((2-(3-methylpyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidine (**45a**)*

Compound **45a** was obtained as a colorless oil (yield 32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53-8.39 (m, 1H), 7.38-7.32 (m, 1H), 7.31 (s, 1H), 7.06-6.97 (m, 1H), 3.94-3.81 (m, 1H), 3.72 (d, *J* = 14.8 Hz, 1H), 3.52 (d, *J* = 15.2 Hz, 1H), 3.36-3.27 (m, 1H), 2.61 (s, 3H), 2.48-2.30 (m, 4H), 2.27-2.16 (m ,1H), 2.13-1.96 (m, 2H), 1.95-1.85 (m, 1H). MS (ESI/APCI) m/z 302.8 $[M+H]^+$. $[\alpha]_D^{25}$ = -2 (c = 0.2, MeOH).

*6.1.23.2. (S)-4-Chloro-2-methyl-6-((2-(4-methylpyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidine (**45b**)*

Compound **45b** was obtained as a colorless oil (yield 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42-8.27 (m, 1H), 7.38-7.25 (m, 1H), 7.20-7.08 (m, 1H), 6.99-6.89 (m, 1H), 3.87-3.63 (m, 2H), 3.59-3.45 (m, 1H), 3.34-3.18 (m, 1H), 2.64 (s, 3H), 2.49-2.37 (m, 1H), 2.37-2.17 (m, 4H), 2.08-1.77 (m, 3H). MS (ESI/APCI) m/z 302.9 $[M+H]^+$. $[\alpha]_D^{25}$ = -3 (c = 0.2, MeOH).

*6.1.23.3. (S)-4-Chloro-2-methyl-6-((2-(5-methylpyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidine (**45c**)*

Compound **45c** was obtained as a colorless oil (yield 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38-8.30 (m, 1H), 7.48-7.40 (m, 1H), 7.38-7.29 (m, 2H), 3.80 (d, *J* = 16.0 Hz, 1H), 3.74-3.63 (m, 1H), 3.49 (d, *J* = 16.0 Hz, 1H), 3.31-3.20 (m, 1H), 2.63 (s, 3H), 2.43-2.36 (m, 1H), 2.34-2.22 (m ,4H), 2.08-1.96 (m,

1H), 1.95-1.85 (m, 2H). MS (ESI/APCI) m/z 302.9 $[M+H]^+$. $[\alpha]_D^{25}$ = -6 (c = 0.2, MeOH).

*6.1.23.4. (S)-4-Chloro-2-methyl-6-((2-(6-methylpyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidine (**45d**)*

Compound **45d** was obtained as a colorless oil (yield 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56-7.48 (m, 1H), 7.36 (s, 1H), 7.30 (d, *J* = 7.6 Hz, 1H), 6.99 (d, *J* = 7.6 Hz, 1H), 3.83 (d, *J* = 16.0 Hz, 1H), 3.75-3.65 (m, 1H), 3.49 (d, *J* = 16.0 Hz, 1H), 3.31-3.20 (m, 1H), 2.63 (s, 3H), 2.52 (s, 3H), 2.45-2.29 (m, 2H), 2.05-1.93 (m, 1H), 1.93-1.78 (m, 2H). MS (ESI/APCI) m/z 303.0 $[M+H]^+$. $[\alpha]_D^{25}$ = +2 (c = 0.2, MeOH).

*6.1.24. General procedure for the synthesis of **46-49***

A mixture of **45a-d** (0.20 mmol, 1.0 eq), *N*-methylpiperazine (1.0 mmol, 5.0 eq) and TEA (2.0 mmol, 10 eq) in ethanol (5 mL) was stirred at 85 ^{o}C for 3 h. The mixture was concentrated and purified by basic Al_2O_3 column chromatography (dichloromethane/methanol = 100/1) to give the desired product.

6.1.24.1.

*(S)-2-Methyl-4-(4-methylpiperazin-1-yl)-6-((2-(3-methylpyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidine (**46**)*

Compound **46** was obtained as a colorless oil (yield 23%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53-8.41 (m, 1H), 7.37-7.30 (m, 1H), 7.06-6.96 (m, 1H), 6.42 (s, 1H), 3.99-3.81 (m, 1H), 3.73-3.50 (m, 5H), 3.48-3.29 (m, 2H), 2.53-2.38 (m, 8H), 2.36-2.30 (m, 6H), 2.25-2.14 (m, 1H), 2.11-2.00 (m, 2H), 1.93-1.82 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.4, 166.3, 162.5, 159.9, 147.3, 138.0, 131.7, 121.7, 98.3, 67.1, 60.6, 54.8, 54.7, 46.2, 43.7, 31.4, 26.0, 23.1, 18.8. HRMS (ESI): calcd for $C_{21}H_{30}N_6$ $[M+H]^+$ 367.2605, found 367.2608. Purity: 99.4%. $[\alpha]_D^{25}$ = +13 (c = 0.2, MeOH).

6.1.24.2.

*(S)-2-Methyl-4-(4-methylpiperazin-1-yl)-6-((2-(4-methylpyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidine (**47**)*

Compound **47** was obtained as a colorless oil (yield 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (d, *J* = 4.8 Hz, 1H), 7.26-7.23 (m, 1H), 6.93 (d, *J* = 4.4 Hz, 1H), 6.46 (s, 1H), 3.74-3.58 (m, 6H), 3.50-3.40 (m, 1H), 3.39-3.29 (m, 1H), 2.54-2.43 (m, 8H), 2.36 (s, 3H), 2.30 (s, 3H), 2.05-1.96 (m, 1H), 1.93-1.86 (m, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.7, 166.6, 163.1, 162.6, 148.8, 147.6, 123.0, 122.5, 98.1, 71.0, 60.4, 54.8, 54.7, 46.2, 43.6, 33.4, 26.0, 23.2, 21.2. HRMS (ESI): calcd for $C_{21}H_{30}N_6$ $[M+H]^+$ 367.2605, found 367.2609. Purity: 97.7%. $[\alpha]_D^{25}$ = +5 (c = 0.2, MeOH).

6.1.24.3.

*(S)-2-Methyl-4-(4-methylpiperazin-1-yl)-6-((2-(5-methylpyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidine (**48**)*

Compound **48** was obtained as a colorless oil (yield 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.41 (d, *J* = 7.6 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 6.46 (s, 1H), 3.71-3.54 (m, 6H), 3.37-3.25 (m, 2H), 2.54-2.38 (m, 8H), 2.34 (s, 3H), 2.31-2.24 (m, 4H), 2.05-1.94 (m, 1H), 1.89-1.78 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.8, 166.7, 162.7, 160.4, 149.6, 137.2, 131.4, 121.1, 97.9, 70.8, 60.3, 54.8, 54.6, 46.3, 43.8, 33.4, 26.1, 23.1, 18.2. HRMS (ESI): calcd for $C_{21}H_{30}N_6$ $[M+H]^+$ 367.2605, found 367.2609. Purity: 98.8%. $[\alpha]_D^{25}$ = +2 (c = 0.2, MeOH).

6.1.24.4.

*(S)-2-Methyl-4-(4-methylpiperazin-1-yl)-6-((2-(6-methylpyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidine (**49**)*

Compound **49** was obtained as a colorless oil (yield 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53-7.47 (m, 1H), 7.31 (d, *J* = 7.6 Hz, 1H), 6.97 (d, *J* = 7.6 Hz, 1 H), 6.48 (s, 1H), 3.74-3.60 (m, 6H), 3.38-3.20 (m, 2H), 2.51 (s, 3H), 2.49-2.30 (m, 8H), 2.30-2.26 (m, 4H), 1.99-1.75 (m, 3H). ^{13}C NMR (150 MHz,

$CDCl_3$) δ 166.7, 166.6, 162.9, 162.6, 157.5, 136.9, 121.6, 118.1, 97.9, 71.1, 60.1, 54.7, 54.4, 46.2, 43.7, 33.6, 26.0, 24.5, 23.1. HRMS (ESI): calcd for $C_{21}H_{30}N_6$ $[M+H]^+$ 367.2605, found 367.2609. Purity: 98.9%. $[\alpha]_D^{25}$ = +6 (c = 0.2, MeOH).

*6.1.25. Procedure for the synthesis of **46-salt***

Compound **46** (1.1 g, 3.0 mmol) was added in one portion to a solution of D-tartaric acid (1.7 g, 11 mmol) in IPA/EA (10 mL/40 mL) at 60 °C for 10 min. The reaction was cooled to room temperature naturally and filtrated. The filter cake was dried in vacuum to give the desired product (2.0 g, 83%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (d, *J* = 3.6 Hz, 1H), 7.57 (d, *J* = 7.6 Hz, 1H), 7.30-7.18 (m, 1H), 6.56 (s, 1H), 4.50-4.38 (m, 1H), 4.24 (s, 6H), 3.90-3.71 (m, 2H), 3.66-3.43 (m, 5H), 2.97-2.77 (m, 1H), 2.53 (s, 4H), 2.42-2.20 (m, 10H), 2.07-1.93 (m, 2H), 1.93-1.81 (m, 1H). Purity: 99.0%.

*6.1.26. General procedure for the synthesis of **53-55***

A mixture of **45a** (0.10 mmol, 1.0 eq), corresponding amine (0.50 mmol, 5.0 eq) and TEA (1.0 mmol, 10 eq) in ethanol (5 mL) was stirred at 85 °C for 3 h. The mixture was concentrated and purified by basic Al_2O_3 column chromatography (dichloromethane/methanol = 100/1) to give the desired product.

6.1.26.1.

*(S)-1-Methyl-4-(2-methyl-6-((2-(3-methylpyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidin-4-yl)-1,4-diazepane (**53**)*

Compound **53** was obtained as a yellow oil (yield 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, *J* = 4.0 Hz, 1H), 7.33 (d, *J* = 7.2 Hz, 1H), 7.05-6.93 (m, 1H), 6.29 (s, 1H), 3.98-3.19 (m, 8H), 2.70-2.46 (m, 5H), 2.43-2.30 (m, 9H), 2.24-2.12 (m, 1H), 2.09-1.79 (m, 5H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.2, 165.7, 162.0, 159.6, 147.2, 138.1, 131.8, 121.7, 97.7, 67.3, 60.4, 58.3, 57.5, 54.5, 46.6, 45.8, 45.2, 31.2, 27.3, 26.1, 23.1, 18.7. HRMS (ESI): calcd for $C_{22}H_{32}N_6$ $[M+H]^+$ 381.2761, found 381.2773. Purity: 99.1%. $[\alpha]_D^{25}$ = +8 (c = 0.2, MeOH).

6.1.26.2.

*(S)-N,N-Dimethyl-1-(2-methyl-6-((2-(3-methylpyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidin-4-yl)piperidin-4-amine (**54**)*

Compound **54** was obtained as a yellow oil (yield 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (d, *J* = 4.0 Hz, 1H), 7.30 (d, *J* = 7.6 Hz, 1H), 7.00-6.92 (m, 1H), 6.36 (s, 1H), 4.47-4.27 (m, 2H), 3.81 (t, *J* = 8.0 Hz, 1H), 3.61 (d, *J* = 14.4 Hz, 1H), 3.36-3.26 (m, 2H), 2.79-2.64 (m, 2H), 2.42-2.28 (m, 8H), 2.25 (s, 6H), 2.18-1.76 (m, 6H), 1.44-1.29 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.4, 166.1, 162.1, 159.6, 147.2, 137.9, 131.6, 121.6, 98.1, 67.0, 62.3, 60.5, 54.6, 43.2, 43.1, 41.6, 31.2, 28.1, 28.0, 26.0, 23.0, 18.7. HRMS (ESI): calcd for $C_{23}H_{34}N_6$ $[M+H]^+$ 395.2918, found 395.2926. Purity: 99.5%. $[\alpha]_D^{25}$ = +17 (c = 0.2, MeOH).

6.1.26.3.

*(S)-2-Methyl-6-((2-(3-methylpyridin-2-yl)pyrrolidin-1-yl)methyl)-N-(2-morpholinoethyl)pyrimidin-4-amine (**55**)*

Compound **55** was obtained as a yellow oil (yield 38%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, *J* = 4.0 Hz, 1H), 7.33 (d, *J* = 7.6 Hz, 1H), 7.02-6.93 (m, 1H), 6.30 (s, 1H), 5.42 (s, 1H), 3.84 (t, *J* = 8.0 Hz, 1H), 3.74-3.65 (m, 4H), 3.60 (d, *J* = 14.8 Hz, 1H), 3.40-3.15 (m, 4H), 2.53 (t, *J* = 6.0 Hz, 2H), 2.50-2.41 (m, 4H), 2.39 (s, 3H), 2.36-2.29 (m, 4H), 2.22-1.80 (m, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.6, 165.8, 162.7, 159.3, 147.3, 138.0, 131.8, 121.7, 99.0, 66.9, 59.8, 56.8, 54.4, 53.2, 37.2, 31.2, 25.8, 23.0, 18.7. HRMS (ESI): calcd for $C_{22}H_{32}N_6O$ $[M+H]^+$ 397.2710, found 397.2725. Purity: 99.8%. $[\alpha]_D^{25}$ = +15 (c = 0.2, MeOH).

6.1.29.

*(S)-5-Fluoro-2-methyl-4-(4-methylpiperazin-1-yl)-6-((2-(3-methylpyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidine (**50**)*

A mixture of **44a** (24 mg, 0.15 mmol), 5-fluoro-4-(fluoromethyl)-2-methyl-6-(4-methylpiperazin-1-yl)pyrimidine (24 mg, 0.15 mmol), 2-ethyl-2-(hydroxymethyl)propane-1,3-diol (53 mg, 0.3 mmol) and KI (8.3 mg, 0.050 mmol) in H_2O (2 mL) was stirred at 120 ^oC overnight. The mixture was concentrated and purified by basic Al_2O_3 column chromatography (dichloromethane/methanol = 100/1) to give the desired product (20 mg, 35%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 8.42 (d, *J* = 3.6 Hz, 1H), 7.33 (d, *J* = 7.6 Hz, 1H), 7.03-6.94 (m, 1H), 3.87 (t, *J* = 8.0 Hz, 1H), 3.77-3.64 (m, 5H), 3.54-3.45 (m, 1H), 3.33-3.23 (m, 1H), 2.55-2.47 (m, 1H), 2.47-2.41 (m, 4H), 2.38 (s, 3H), 2.36 (s, 3H), 2.30 (s, 3H), 2.19-2.08 (m, 1H), 2.05-1.95 (m, 1H), 1.93-1.76 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.1 (d, *J* = 7.5 Hz), 159.6, 151.1 (d, *J* = 4.5 Hz), 150.6 (d, *J* = 15 Hz), 147.2, 144.1 (d, *J* = 256.5 Hz), 137.8, 131.5, 121.5, 66.3, 55.1, 54.1, 52.4, 46.1, 45.9 (d, *J* = 7.5 Hz), 31.4, 25.3, 22.7, 18.6. HRMS (ESI): calcd for $C_{21}H_{29}FN_6$ $[M+H]^+$ 385.2510, found 385.2518. Purity: 99.1%. $[\alpha]_D^{25}$ = -3 (c = 0.2, MeOH).

6.1.30. *tert-Butyl (S)-4-(5-cyano-2-methyl-6-((2-(3-methylpyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidin-4-yl)piperazine-1-carboxylate (**51**)*

A mixture of **44a** (32 mg, 0.20 mmol), *tert*-butyl 4-(6-(chloromethyl)-5-cyano-2-methylpyrimidin-4-yl)piperazine-1-carboxylate (70 mg, 0.20 mmol), KI (3.3 mg, 0.020 mmol), and DIPEA (65 mg, 0.50 mmol) in acetonitrile (5 mL) was stirred at room temperature overnight. The mixture was concentrated and purified by basic Al_2O_3 column chromatography (petroleum ether/ethyl acetate = 1/1) to give the desired product (60 mg, 63%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.57-7.36 (m, 1H), 7.18-6.97 (m, 1H), 4.24-3.67 (m, 7H), 3.63-3.38 (m, 5H), 2.56-2.27 (m, 7H), 2.12-2.01 (m, 2H), 2.00-1.92 (m, 2H), 1.48 (s, 9H). MS (ESI/APCI) m/z 477.9 $[M+H]^+$.

6.1.31.

*(S)-2-Methyl-4-(4-methylpiperazin-1-yl)-6-((2-(3-methylpyridin-2-yl)pyrrolidin-1-yl)methyl)pyrimidine-5-carbonitrile (**52**)*

A mixture of **51** (57 mg, 0.12 mmol) and TFA (1 mL) in DCM (2 mL) was stirred at room temperature for 2 h. The mixture was concentrated in vacuum to give the residue. To a solution of this crude product in methanol (2 mL) was added formaldehyde (37 wt percent in water, 0.5 mL) and $NaBH_3CN$ (15 mg, 0.24 mmol). The reaction was stirred at room temperature for 2 h, and quenched with saturated $NaHCO_3$ aqueous solution (5 mL). The aqueous layer was extracted with dichloromethane (30 mL * 3), dried over Na_2SO_4, filtered and concentrated. The residue was purified by basic Al_2O_3 column chromatography (petroleum ether/ethyl acetate = 1/10) to give the desired product (30 mg, 64%) as a yellow oil. ^1H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, *J* = 3.6 Hz, 1H), 7.34 (d, *J* = 7.6 Hz, 1H), 7.04-6.94 (m, 1H), 4.05-3.96 (m, 1H), 3.95-3.86 (m, 4H), 3.82 (d, *J* = 13.2 Hz, 1H), 3.63 (d, *J* = 13.2 Hz, 1H), 3.44-3.34 (m, 1H), 2.64-2.54 (m, 1H), 2.51-2.45 (m, 4H), 2.43 (s, 3H), 2.40 (s, 3H), 2.31 (s, 3H), 2.34-2.12 (m, 1H), 2.10-1.81 (m, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.2, 168.3, 161.8, 159.3, 147.2, 138.0, 131.7, 121.7, 117.0, 87.5, 67.0, 57.8, 54.9, 54.5, 46.3, 46.0, 31.3, 26.4, 23.0, 18.8. HRMS (ESI): calcd for $C_{22}H_{29}N_7$ $[M+H]^+$ 392.2557., found 392.2564. Purity: 98.9%. $[\alpha]_D^{25}$ = +3 (c = 0.2, MeOH).

6.2. ***In vitro* biological assays**

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

6.2.2. FLIPR Tetra calcium mobilization assay

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 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+} T-Cells were isolated from human whole blood and subsequently activated and expanded using a CD3/CD28 expansion kit (Life Technologies). The cells were frozen in ThermoFisher-formulated Recovery Cell Culture Freezing Medium containing 10% Dimethyl sulfoxide (DMSO) and 10% Fetal Bovine Serum (FBS) (ThermoFisher Catalog No. 10100147). When used, cells were resuspended using room temperature 1X HBSS/20 mM HEPES/0.005% P-104 assay buffer, adjusted the volume of the suspension to achieve a cell concentration of 2.5×10^6 cells/mL. 2X Calcium 4 dye (20 µL/well) were added and the mixture were centrifuged briefly (~10 s) and stopped when it reached 1000 rpm. The plates were allowed to equilibrate before compounds and CXCL12 were added to the plates. The raw data were analyzed using Abase. The percent (%) effect at each concentration of compound was calculated by Abase and was based on and relative to the amount of calcium produced in the positive and negative control wells contained within each assay plate. The concentrations and % effect values for tested compounds were plotted by Abase and the concentration of compound required for 50% effect (IC_{50}) was determined with a four-parameter logistic dose response equation.

6.2.3. Matrigel invasion assay

The human breast cancer cell line MDA-MB-231 was purchased from ATCC (Manassas, VA). MDA-MB-231 cell 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 ºC

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 (1×10^5 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.

6.2.4. Cell viability (MTT) assay

Cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Briefly, cells were plated at a density of 5×10^3 cells/well on 96-well plates and subjected to compound **3** treatment. Following 48 h incubation at 37 ºC in a humidified atmosphere containing 5% CO_2/95% air, the samples were incubated for another 4 h with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reagent. The formazan product was dissolved in dimethyl sulfoxide and read at 570 nm on a Victor3 Multi Label plate reader (PerkinElmer, Boston, MA, USA).

6.2.5. Other assays

CXCR1 functional assay, CCR6 functional assay, these assay procedures have been reported previously[29].

6.3. *Preliminary in vitro safety test*

CYP inhibitory potency and human/mouse liver microsomes metabolic stability were evaluated as previously reported [39]. hERG inhibition and Caco-2 assays were reported before [40].

6.4. *In vivo tumor metastasis test*

Red fluorescence protein (RFP)-labeled lung carcinoma LL/2 cells (4×10^5 cells) were injected into C57BL/6 mice in a volume of 200 μL PBS via the tail intravenous injection. Vehicle, compound **14** (70 mg/kg) were intraperitoneal injected to these mice 30min before tumor cells injection, and 3 h after tumor cells injection. Then, these mice were given the same dose of vehicle, compound **14** (70 mg/kg) every day for 18 days. Eighteen days after injection of tumor cells, mice were sacrificed and lung tissues were collected. The lung metastasis of LL/2 cells in mice was analyzed by measuring RFP fluorescence intensities with the PerkinElmer IVIS Lumina II.

6.5 *Procedure for pKa and log $D_{7.4}$ measurements*

The pKa was determined by the automated Sirius T3 system. The Sirius T3 can measure one sample in around 4 min, using 5 μL of 10 mM DMSO stock solution and collecting up to 50 data points from pH 2.0 to pH 12.0 by UV detection.

The 1-octanol/water partition coefficient at $pH_{7.4}$ was determined using the shake flask technique. A

phosphate $pH_{7.4}$ aqueous buffer solution has been prepared and then saturated with n-octanol. Likewise, an n-octanol solution saturated with $pH_{7.4}$ aqueous buffer has been also prepared. Approximately 1 mg of sample was dispensed into a 3 ml glass screw-top vial to which 1 ml octanol was added. Compounds that dissolved incompletely were diluted and further sonicated until completely dissolved. A final volume of 1ml of octanol solution and 1 ml of phosphate buffer (PBS) $pH_{7.4}$ were shaken for 24 h. The resulting solution was centrifuged and the octanol and water layers were extracted separated. The amount of sample present in the octanol and PBS solutions was determined by HPLC. The $logD_{7.4}$ was calculated from the area under the curve obtain from the 2 samples, corrected for the dilution factors and injection volume. Results shown are average value of three independent experiments.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at

References

[1] 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.

[2] 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.

[3] T. Pozzobon, G. Goldoni, A. Viola, B. Molon, CXCR4 signaling in health and disease, Immunol. Lett. 177 (2016) 6-15.

[4] N. Nagarsheth, M.S. Wicha, W. Zou, Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy, Nat. Rev. Immunol. 17 (2017) 559-572.

[5] B. Debnath, S. Xu, F. Grande, A. Garofalo, N. Neamati, Small molecule inhibitors of CXCR4, Theranostics 3 (2013) 47-75.

[6] 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.

[7] F. Grande, G. Giancotti, G. Ioele, M.A. Occhiuzzi, A. Garofalo, An update on small molecules targeting CXCR4 as starting points for the development of anti-cancer therapeutics, Eur. J. Med. Chem. 139 (2017) 519-530.

[8] H. Zhang, D. Kang, B. Huang, N. Liu, F. Zhao, P. Zhan, X. Liu, Discovery of non-peptide small molecular CXCR4 antagonists as anti-HIV agents: Recent advances and future opportunities, Eur. J. Med. Chem. 114 (2016) 65-78.

[9] S. Hotte, H. Hirte, A. Iacobucci, D. Wong, L. Cantin, W. Korz, W. Miller, Phase I/II study of CTCE-9908, a novel anticancer agent that inhibits CXCR4, in patients with advanced solid cancers, Mol. Cancer Ther. 6 (2007) A153-A153.

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

[11] S.P. Fricker, V. Anastassov, J. Cox, M.C. Darkes, O. Grujic, S.R. Idzan, J. Labrecque, G. Lau, R.M. Mosi, K.L. Nelson, L. Qin, Z. Santucci, R.S. Wong, Characterization of the molecular pharmacology of AMD3100: a specific antagonist of the G-protein coupled chemokine receptor, CXCR4, Biochem. Pharmacol. 72 (2006) 588-596.

[12] E. De Clercq, The AMD3100 story: the path to the discovery of a stem cell mobilizer (Mozobil), Biochem. Pharmacol. 77 (2009) 1655-1664.

[13] J.F. DiPersio, G.L. Uy, U. Yasothan, P. Kirkpatrick, Plerixafor, Nat. Rev. Drug Discov. 8 (2009) 105-106.

[14] K. Jung, T. Heishi, J. Incio, Y. Huang, E.Y. Beech, M. Pinter, W.W. Ho, K. Kawaguchi, N.N. Rahbari, E. Chung, J.K. Kim, J.W. Clark, C.G. Willett, S.H. Yun, A.D. Luster, T.P. Padera, R.K. Jain, D. Fukumura, Targeting CXCR4-dependent immunosuppressive Ly6C(low) monocytes improves antiangiogenic therapy in colorectal cancer, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) 10455-10460.

[15] Y. Chen, R.R. Ramjiawan, T. Reiberger, M.R. Ng, T. Hato, Y. Huang, H. Ochiai, S. Kitahara, E.C. Unan, T.P. Reddy, C. Fan, P. Huang, N. Bardeesy, A.X. Zhu, R.K. Jain, D.G. Duda, CXCR4 inhibition in tumor microenvironment facilitates anti-programmed death receptor-1 immunotherapy in sorafenib-treated hepatocellular carcinoma in mice, Hepatology 61 (2015) 1591-1602.

[16] F. Grande, I. Barone, F. Aiello, A. Brancale, M. Cancellieri, M. Badolato, F. Chemi, C. Giordano, V. Vircillo, D. Bonofiglio, A. Garofalo, S. Andò, S. Catalano, Identification of novel 2-(1H-indol-1-yl)-benzohydrazides CXCR4 ligands impairing breast cancer growth and motility, Future Med. Chem. 8 (2016) 93-106.

[17] 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.

[18] 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.

[19] 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.

[20] 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.

[21] 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.

[22] 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.
[23] 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.
[24] 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.
[25] J.B. Crawford, G. Chen, D. Gauthier, T. Wilson, B. Carpenter, I.R. Baird, E. McEachern, A. Kaller, C. Harwig, B. Atsma, R.T. Skerlj, G.J. Bridger, AMD070, a CXCR4 Chemokine Receptor Antagonist: Practical Large-Scale Laboratory Synthesis, Org. Process Res. Dev. 12 (2008) 823-830.
[26] S. Boggs, V.I. Elitzin, K. Gudmundsson, M.T. Martin, M.J. Sharp, Kilogram-Scale Synthesis of the CXCR4 Antagonist GSK812397, Org. Process Res. Dev. 13 (2009) 781-785.
[27] R.J. Wilson, E. Jecs, E.J. Miller, H.H. Nguyen, Y.A. Tahirovic, V.M. Truax, M.B. Kim, K.M. Kuo, T. Wang, C.S. Sum, M.E. Cvijic, A.A. Paiva, G.M. Schroeder, L.J. Wilson, D.C. Liotta, Synthesis and SAR of 1,2,3,4-Tetrahydroisoquinoline-Based CXCR4 Antagonists, ACS Med. Chem. Lett. 9 (2018) 17-22.
[28] 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.
[29] 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.
[30] T.J. Dickerson, K.D. Janda, Aqueous aldol catalysis by a nicotine metabolite, J. Am. Chem. Soc. 124 (2002) 3220-3221.
[31] I.R. Baxendale, G. Brusotti, M. Matsuoka, S.V. Ley, Synthesis of nornicotine, nicotine and other functionalised derivatives using solid-supported reagents and scavengers, J. Chem. Soc., Perkin Trans. 1 (2002) 143-154.
[32] P.A. Champagne, A. Saint-Martin, M. Drouin, J.F. Paquin, Triol-promoted activation of C-F bonds: Amination of benzylic fluorides under highly concentrated conditions mediated by 1,1,1-tris(hydroxymethyl)propane, Beilstein J Org Chem 9 (2013) 2451-2456.
[33] 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.
[34] P.S. Charifson, W.P. Walters, Acidic and basic drugs in medicinal chemistry: a perspective, J. Med. Chem. 57 (2014) 9701-9717.
[35] N. Colclough, L. Ruston, J.M. Wood, P.A. MacFaul, Species differences in drug plasma protein binding, Med. Chem. Commun. 5 (2014) 963-967.
[36] U. Kragh-Hansen, Molecular aspects of ligand binding to serum albumin, Pharmacol. Rev. 33 (1981) 17-53.
[37] D.A. Smith, K. Beaumont, T.S. Maurer, L. Di, Clearance in Drug Design, J. Med. Chem. 62 (2019) 2245-2255.
[38] J. Hou, J. Ju, Z. Zhang, C. Zhao, Z. Li, J. Zheng, T. Sheng, H. Zhang, L. Hu, X. Yu, W. Zhang, Y. Li, M. Wu, H. Ma, X. Zhang, S. He, Discovery of potent necroptosis inhibitors targeting RIPK1 kinase

activity for the treatment of inflammatory disorder and cancer metastasis, Cell Death Dis. 10 (2019) 493.

[39] 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.

[40] 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.

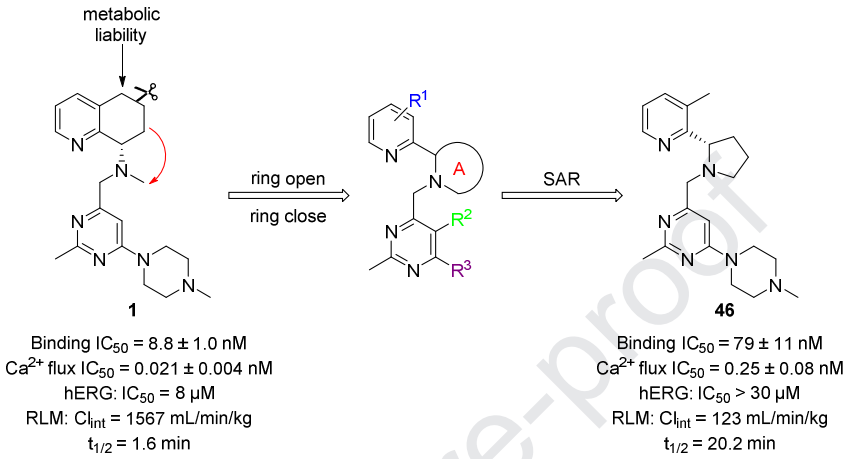
Highlights

(1) Structural optimization of pyrrolidine -based CXCR4 antagonists were reported.
(2) Compound **46** competes with APC-conjugate 12G5 for CXCR4 binding (IC_{50} = 79 nM).
(3) Compound **46** inhibits CXCL12 induced cytosolic calcium increase (IC_{50} = 0.25 nM).
(4) Compound **46** exhibits improved metabolic stability in rat and human liver microsomes.
(5) Compound **46** demonstrates marked efficacy in a cancer metastasis model in mice.

Graphical Abstract

Design, synthesis, and evaluation of pyrrolidine based CXCR4 antagonists with *in vivo* anti-tumor metastatic activity

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



Compound **46** is a potent CXCR4 antagonist with significantly improved metabolic stability in rat and human liver microsomes. Moreover, **46** potently inhibits CXCR4/CXLC12 mediated chemotaxis in a matrigel invasion assay, exhibits good physicochemical properties and *in vitro* safety profiles. Importantly, compound **46** demonstrates marked efficacy in a cancer metastasis model in mice.

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: