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Design and Synthesis of Potent, Selective Inhibitors of Protein Arginine Methyltransferase 4 Against Acute Myeloid Leukemia

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ABSTRACT

PRMT4 is a type I protein arginine methyltransferase and plays important roles in various cellular processes. Overexpression of PRMT4 has been found to be involved in several types of cancers. Selective and in vivo effective PRMT4 inhibitors are needed for demonstrating PRMT4 as a promising therapeutic target. Based on compound **6**, a weak dual PRMT4/6 inhibitor, we constructed a tetrahydroisoquinoline scaffold through a cut-and-sew scaffold hopping strategy. The subsequent SAR optimization efforts employed structure-based approach led to the identification of a novel PRMT4

inhibitor **49**. Compound **49** exhibited prominently high potency and selectivity, moderate pharmacokinetic profiles and good anti-tumor efficacy in acute myeloid leukemia xenograft model via oral administration, thus demonstrating this compound as a useful pharmacological tool for further target validation and drug development in cancer therapy.

Keywords: PRMT4; structure-based drug discovery; scaffold hopping; acute myeloid leukemia

INTRODUCTION

Post-translational modification (PTM) of protein is an economical machinery in biology to dynamically regulate a broad range of cellular processes covering cellular metabolism, transcription, protein translation, and signal transduction.[1] In the past two decades, various proteins involved in PTM were discovered[2] and the enzymes responsible for the methylation on the guanidinium group of arginine are known as protein arginine methyltransferases (PRMTs), which utilize S-adenosyl methionine (SAM) as the methyl donor[3] to perform the catalysis. Nine PRMTs have been identified in human genome and divided into three subfamilies in term of the degree and position of methylation.[4, 5] Type I enzymes including PRMT1, 2, 3, 4, 6 and 8 could transfer one methyl group to arginine to convert it into monomethylarginine (MMA) or further add another methyl group to the same nitrogen atom to become asymmetric dimethylarginine (aDMA). Type II enzymes, consisting of PRMT5 and 9, generate MMA or symmetric dimethylarginine (sDMA) , which means one more methyl group

is added to another nitrogen atom of arginine. While PRMT7 is the sole type III enzyme that only produces MMA. [6]

A type I enzyme PRMT4, also known as coactivator-associated arginine methyltransferase 1 (CARM1), was first identified as a transcriptional regulator.[7] PRMT4 can function as a transcriptional coactivator of nuclear receptors and methylates steroid receptor coactivators including SRC3 and CBP/P300.[8, 9] Furthermore, PRMT4 regulates gene expression by multiple mechanisms. Specifically, PRMT4 positively regulates transcription by methylating histone H3 at arginine 17 and 26.[10, 11] PRMT4 also methylates the RNA-binding proteins PABP1[12] , HuR[13] and HuD[14] to affect their ability to bind to the transcription-related proteins, and methylates splicing factors such as CA150 [15] to regulate the exon skipping.

Overexpression of PRMT4 has been demonstrated in various cell lines of hematologic cancers and solid tumors, such as leukemia,[16, 17] breast,[18] prostate,[19] liver[20] and colorectal [21, 22] cancers. Wang et al. identified that PRMT4 catalyzed the methylation of BAF155, a critical component of SWI/SNF chromatin remodeling complex, and therefore enhanced the tumor progression and metastasis of breast cancer.[23] Moreover, PRMT4 has been found upregulated in grade-3 breast tumors,[24] and the knockdown of PRMT4 inhibited prostate cancer cell proliferation by induction of apoptosis.[25] Recently, an essential role of PRMT4 in myeloid leukemogenesis has been linked to the oncogenic transcription factors which has little effect on normal hematopoiesis. Therefore, PRMT4 is considered to be a promising therapeutic target for anticancer drug development.In past decade, as the increased interest in drug targeting PRMT

enzymes, numerous PRMT4 inhibitors have been reported, and most of them are pan-type I PRMTs inhibitors, which usually are associated with potent activities towards PRMT1, PRMT4 and PRMT6.[26-28] Recent years, selective PRMT4 inhibitors are occasionally reported.[29-31] Purandare et al. from Bristol-Myyers Squibb and Allan et al. from MethylGene reported two series of selective PRMT4 inhibitors sharing alanine amide motif as key pharmacophore. These compounds showed two-digit nanomolar enzymatic activities but with very low cellular activities. Subsequent efforts on developing selective PRMT4 inhibitors were conducted but with no obvious improvement, probably attributed to the poor permeability and metabolic instability that limited the further exploitation.[32-35] In 2017, Drew et al. from Epizyme reported the first oral active PRMT4 inhibitor, EZM2302, which contained 1-amino-3-phenoxypropan-2-ol moiety as arginine mimetic and demonstrated moderate efficacy in multiple myeloma xenograft model.[36] Thus, developing selective PRMT4 inhibitor with in vivo activity is still pressing, which may provide more option for target validation and drug development.

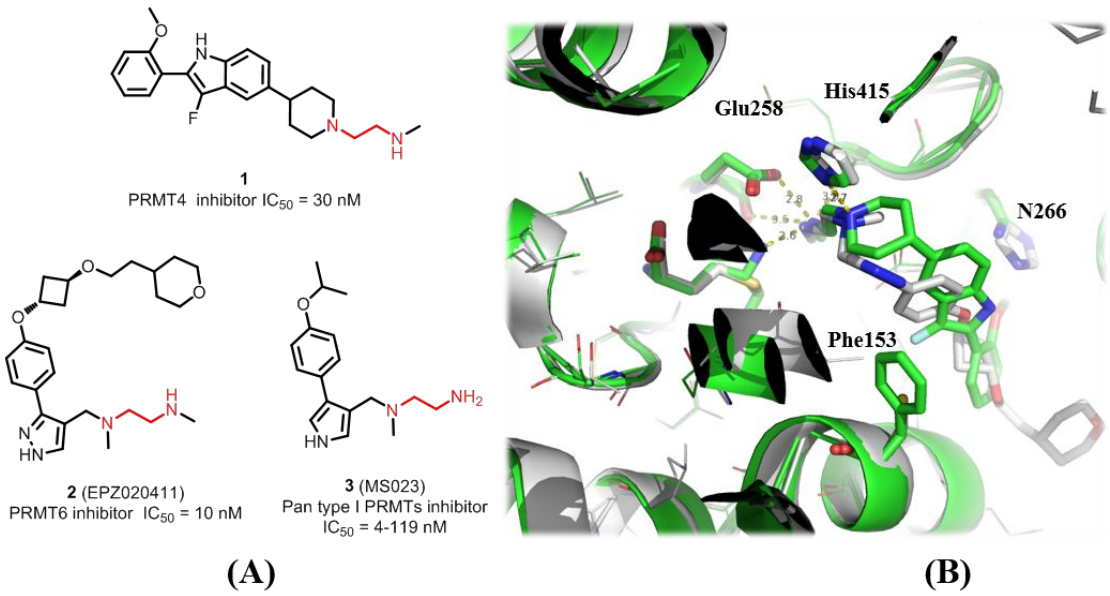


Figure 1. (A) Representative structures and profiles of reported type I PRMT inhibitors. The common moieties of arginine mimetic are highlighted in red. (B) Structural alignment of PRMT inhibitors in complex with PRMT4 (**1**: PDB code 2Y1W) and PRMT6 (**2**: PDB codes 4Y30): **1**(green), **2**(gray).

From the chemical structural point of view, as represented in Figure 1, these inhibitors target the substrate binding pocket by frequently using an ethylenediamino moiety as a warhead to mimic the side chain of arginine residue. Scrutiny of cocrystal structures of compound **1**,[37] EPZ020411 (**2**)[38] nd MS023 (**3**)[27] in complex with PRMT proteins, we find that the ethylenediamino moieties stretched into a narrow subpocket and formed critical hydrogen bonding interactions with two conserved residues (His415 and Glu258 in PRMT4; His317 and Glu155 in PRMT6). However, the entrance of the substrate binding pocket is dynamic and has diversified residues among different PRMTs, which could be utilized to enhance the selectivity towards PRMT4.[27, 39]

In consideration of the ubiquitous expression of type I PRMTs (except for PRMT8)[40] and distinct functions of individual PRMTs [4], improving the selectivity for PRMT4 can

minimize the adverse concerns results from inhibiton of other homologous type I PRMTs. Herein, we report the discovery of (R)-1-(methylamino)-3-(5-((4-(thiazol-2-yl)benzyl)oxy)-3,4-dihydroisoquinolin-2(1*H*) yl)propan-2-ol **49** derived from a previously reported dual PRMT4/6 inhibitor. This potent compound **49** exhibits excellent PRMTs selectivity, moderate pharmacokinetic profiles and high inhibitory efficacy in vitro and in vivo, which will help to validate PRMT4 as a potential therapeutic target and provides a candidate for the treatment of acute myeloid leukemia.

RESULTS AND DISCUSSION

Rational Design of Selective PRMT4 Inhibitor

In order to develop selective PRMT4 inhibitors, we firstly analyzed the essential moiety of arginine mimetic and noticed that a reported alternative 1,3-diaminopropan-2-ol compound **6** could slightly improve the selectivity to PRMT4 (PRMT4 IC_{50} = 1.22 μM; PRMT6, IC_{50} = 3.04 μM), while the ethylenendiamino analogues (compound **4** and **5**) had better activity towards PRMT6 (Figure 2).[39, 41] This implicated that the 1,3-diaminopropan-2-ol may provide new hydrogen bonding pattern to better fit the subpocket of PRMT4. Besides, this aminoalcohol moiety also occurred in the selective PRMT4 inhibitor EZM2302, which further encouraged us to explore selective inhibitor of PRMT4 based on compound **6**. [36] By comparing the crystal structures of PRMT4 with PRMT6, we found that three residues (Phe153, Gln159, and Asn266 in PRMT4 and Cys50, Val56, and His163 in PRMT6) at the entrance of the arginine-binding pocket were not conserved between the two enzymes. In particular, π-π interaction between PRMT4's Phe153 and aromatic ring of inhibitors can further boost the

selectivity in favor of PRMT4 over PRMT6.[34, 35] Combining the aforementioned interaction features, we intended to use the 1,3-diaminopropan-2-ol motif as the warhead and focus the modification on the left part of the inhibitors.

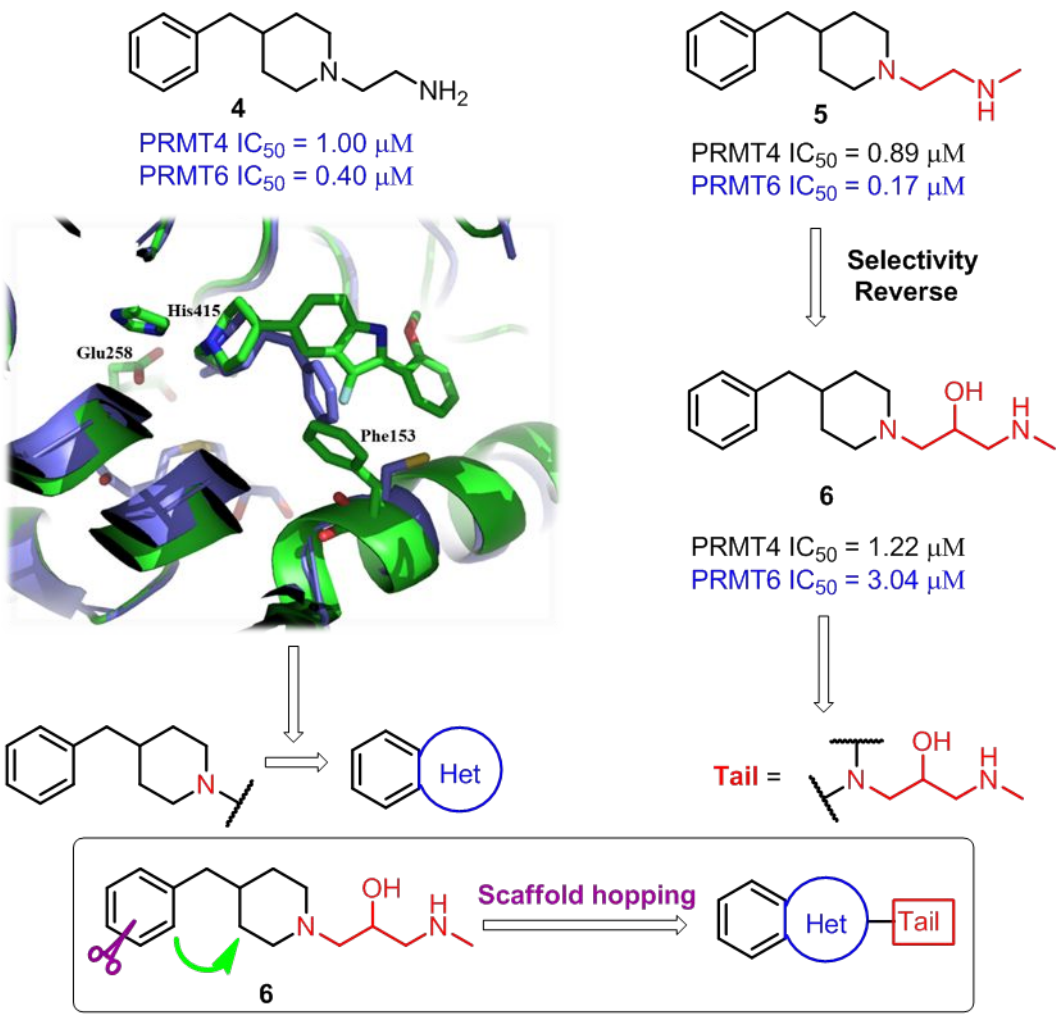
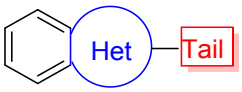


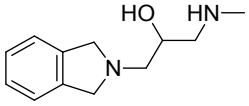
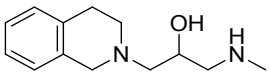
Figure 2. Design strategy for selective PRMT4 inhibitor. Structural alignment of PRMT inhibitors in complex with PRMT4 (**1**: PDB code 2Y1W) and PRMT6 (**4**: PDB codes 5EGS): **1**(green), **4**(bule).

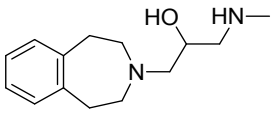
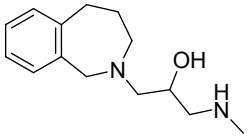
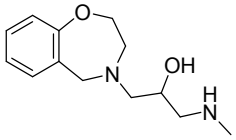
Following the design strategy outline in Figure 2, we initialized the project with scaffold hopping approach by opening and recycling the ring system to generate the bicyclic skeleton, which, as predicted, may enforce the phenyl ring to get closer to Phe153 and form π-π interaction to improve the selectivity and potency for PRMT4 (Figure 2).

With the guidance of this design strategy, compounds **7-11** with varied saturated heterocycle were synthesized. As shown in Table 1, these compounds displayed moderate potency in PRMT4 enzymatic assay except compound **11** that didn't show any inhibition even at 10 μM. Considering the simple structure and preserved activity, compound **7-10** could serve as applicable hit for further optimization. To verify the design stategy and provide rational direction for optimization, we solved the co-crystal structure of compound **10** bound with the catalytic domain of PRMT4 (aa 140-480). As shown in Figure 3, the structure revealed that compound **10** was situated at the substrate binding pocket, and formed critical hydrogen bonding interactions with nearby residues, including Glu258, Met260 and His415. By aligning with the reported crystal structure (PDB code: 2Y1W), we found that the longer tail of compound **10** extended to the position commonly occupied by SAM. Notably, the bicyclic ring of compound **10** had different orientation, facing the residue Met163 and pushing the residue Asn162 away. Importantly, the bicyclic ring of compound **10** was indeed engaged in a T-shape π-π interaction with Phe153 as expected. This distinct binding mode triggered us to further elaborate the SAR of this series of PRMT4 inhibitors.

Table 1. PRMT4 enzymatic activity of compound 7-11



Compd	Structure	IC_{50} (μM)[a]
7		2.46 ± 0.22
8		5.31 ± 1.08

9		8.34 ± 1.16
10		3.19 ± 0.67
11		>10.0

[a] All IC_{50} values are reported as the geometric mean from at least two determinations. The PRMT4 inhibitor MS049 was used as the positive control in our enzymatic assay, exhibiting an IC_{50} value of 32 ± 6 nM.

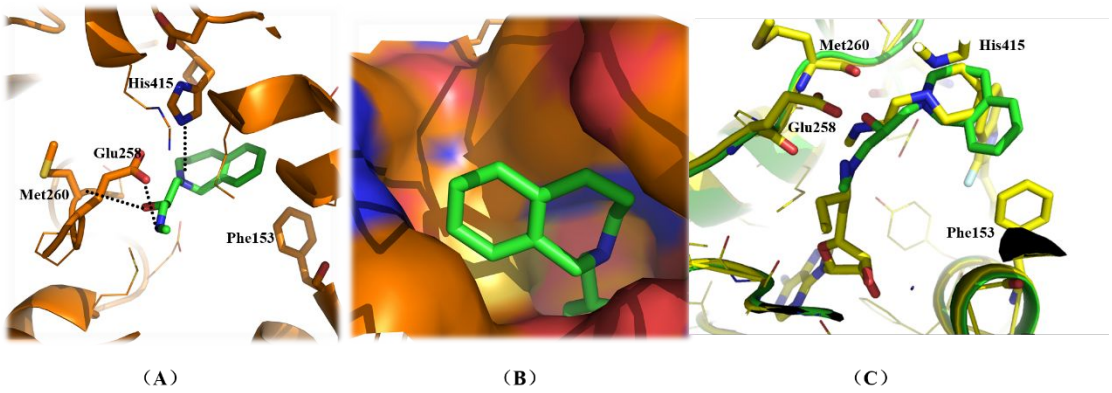


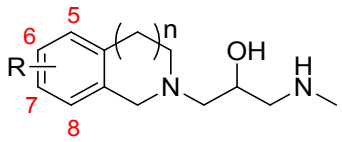
Figure 3. (A) (B) Crystal structures of compounds **10** (green) in complex with PRMT4 (PDB code: 6IZQ). (C) Structural alignment with compound **1** (yellow) (PDB code: 2Y1W).

Lead Optimization

Considering of the synthesis accessibility, we selected compound **8** as the starting point to investigate the substitutions on the phenyl group. According to the cocrystal structure of compound **10** in complex with PRMT4, we could find that the 5- or 6-position at the phenyl group might be the most promising position for extension. Nevertheless, in case of the flexibility of the PRMT4 protein, we synthesized four compounds (**12**-**15**) by using bromide atom as a scanning group at various positions (Table 2). From the result,

we found the 5-position of phenyl group was the possible extension site, as compound **12** displayed higher potency for PRMT4 with an IC_{50} value of 0.130 μM. On the other hand, compound **15** (IC_{50} = 0.772μM) substituted with a bromide atom at 8-position also increased the potency for PRMT4, which was probably attributed to the introduction of halogen bonding interaction between bromide atom and the surrounding residue Tyr154. We also synthesized the 2,3,4,5-tetrahydro-1*H*-benzo[c]azepine derivative **16** with an IC_{50} value of 0.785 μM, which further confirmed the 5-position as the preferred extension site. Furthermore, compound **12** was tested the inhibition for PRMT1 and PRMT6 and it demonstrated excellent selectivity, with more than 450-fold and 320-fold potent for PRMT4 over PRMT1 and PRMT6, respectively (Table 3).Thus, compound **12** was chosen as starting scaffold for the next round SAR study.

Table 2. PRMT4 enzymatic activity of compound 12-16



Compd	R	n	IC_{50} (μM)[a]
12	5-Br	1	0.130 ± 0.012
13	6-Br	1	4.48 ± 0.30
14	7-Br	1	5.22 ± 0.94
15	8-Br	1	0.772 ± 0.028
16	5-Br	2	0.785 ± 0.152

[a] All IC_{50} values are reported as the geometric mean from at least two determinations.

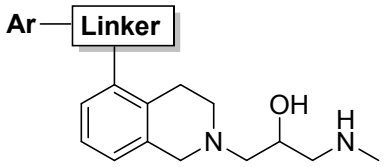
Table 3. Selectivity of compound 12

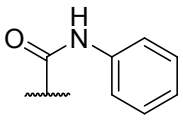
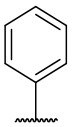
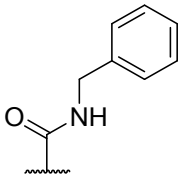
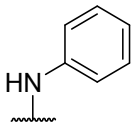
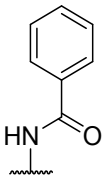
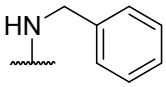
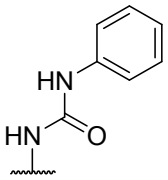
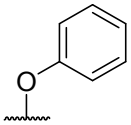
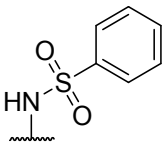
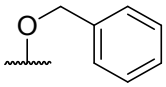
Compd	$IC_{50}(\mu M)$[a]			Selectivity	Selectivity
	PRMT4	PRMT1	PRMT6	Ratio[b] (4vs.1)	Ratio[b] (4vs.6)
12	0.130	58.8	41.2	450	320

[a] All IC_{50} values are reported as the geometric mean from at least two determinations. [b] Selectivity ratio = IC_{50} of PRMTx/ IC_{50} of PRMT4, x=1 or 6.

Taking together the information from the preliminary SAR study and cocrystal structure, we prepared the derivatives with linkers at the 5-position to introduce diverse functional groups (Table 4). The various linkers may direct the aromatic group to different locations and increase potential interactions with the residues at the mouth of the arginine-binding pocket. Without a linker, compound **17** displayed about 4-fold potency loss against PRMT4 comparing with compound **12**. Among analogs with a one-heteroatom linker, amino linkers (**18** and **19**) also resulted in an adverse potency, especially **18** (IC_{50} = 2.11 μM), while ether linkers (**20** and **21**) primarily retained the potency for PRMT4. The benzyl moieties (**19** and **21**) had superior potency over phenyl groups (**18** and **20**), which might benefit from the flexibility of benzyl group adopting the advantageous direction. However, comparing with compound **12**, the synthesized compounds with a two-four heteroatom linker, such as amide (**22**, **23** and **24**), urea (**25**), and sulfamide (**26**), exhibited notably less potent against PRMT4. Nevertheless, the loss of potency of some derivatives could be explained by the disturbance of conserved hydrogen bonding interaction or unbefitting direction of aromatic group. Overall, the ether linker was optimal and showed certain potential for further investigation.

Table 4. PRMT4 enzymatic activity of compound 12, 17-26



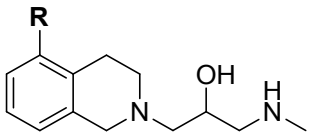
Compd	Linker-Ar	IC_{50}(μ M)[a]	Compd	Linker-Ar	IC_{50}(μ M)[a]
12	Br	0.130 ±0.012	**22**		7.98 ± 1.35
17		0.465 ± 0.055	**23**		2.13 ± 0.16
18		2.11 ± 0.23	**24**		6.12 ± 1.16
19		0.343 ± 0.067	**25**		3.82 ± 0.15
20		0.270 ± 0.041	**26**		4.26 ± 0.12
21		0.165 ± 0.029			

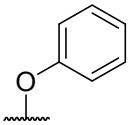
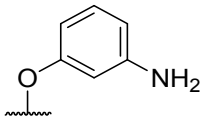
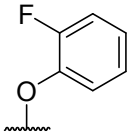
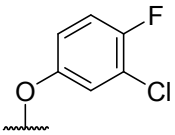
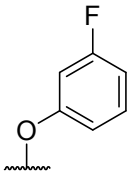
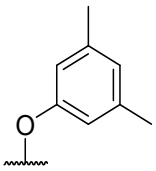
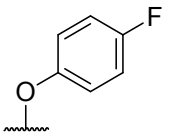
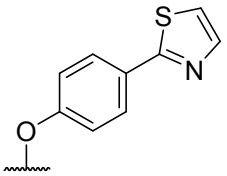
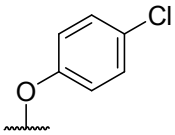
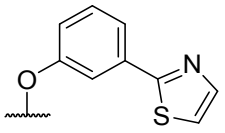
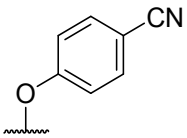
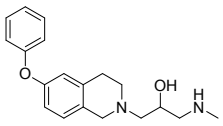
[a] All IC_{50} values are reported as the geometric mean from at least two determinations.

Firstly, we prepared analogs of compound **20** with various substitutes on the additional phenyl group (Table 5). Incorporation of the fluorine atom from the *ortho*-position (**27**) to *meta*- (**28**) or *para*-positions (**29**) led to a significant loss in inhibitory activity. Unfortunately, additional substituents including 4-chlorine (**30**), 4-cyano (**31**), 3-amino (**32**), 3-chloro-4-fluoro (**33**), 3,5-dimethyl (**34**) also suffered from detrimental effect on

inhibition. These results indicate that the phenyl ether scaffold may not be suitable for decorating with simple moieties. On the contrary, aromatic thiazolyl-substituted derivatives (**35** and **36**) maintained the potency against PRMT4, suggesting that additional interaction might be responsible for the retention of activity. To further confirm the influence of extension position, we synthesized the 6-phenyl ether substituted derivative (**37**) of 1,2,3,4-tetrahydroisoquinoline, which showed an IC_{50} value of 0.900 μM （3-fold worse than 5- substituted compound **20**） and indeed exhibited the extension position is important. To understand the differential activities of these compounds, we modeled the binding conformations of compounds **20**, **35** and **37** by utilizing the molecular docking method (Figure S1). The docking result demonstrated that the 1,3-diaminopropan-2-ol motif interacted with protein almost exactly as in the solved co-crystal structure of compound **10**. The 1,2,3,4-tetrahydroisoquinoline scaffold was located at similar position as 2,3,4,5-tetrahydro-1H-benzo[c]azepine of **10**. The 5-substituted phenyl group of **20** extended to the vicinity of residues Tyr150 and Phe153. However, comparing to compound **20**, the 6-substituted phenyl group of **37** reached to solvent-accessible part. Similarly, the phenyl group of compound **35** pointed to the same direction with **20** and the thiazolyl group extended further to the helix structure of residues Tyr150 and Phe153. These modeling confirmed that the 5-position is the right direction for optimization and expounded the boost in activity of compound **35** and **36**.

Table 5. PRMT4 enzymatic activity of compound 20, 27-35



Compd	R	IC_{50}(μ M)[a]	Compd	R	IC_{50}(μ M)[a]
20		0.270 ± 0.041	**32**		3.49 ± 0.08
27		2.07 ± 0.26	**33**		2.05 ± 0.20
28		2.31 ± 0.04	**34**		1.99 ± 0.12
29		2.61 ±0.39	**35**		0.279 ± 0.042
30		1.18 ± 0.30	**36**		0.658 ± 0.105
31		2.31 ± 0.16	**37**[b]		0.900 ± 0.024

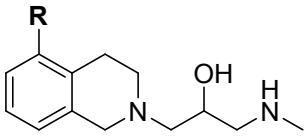
[a] All IC_{50} values are reported as the geometric mean from at least two determinations. [b] Phenyl ether substituted at 6-position of 1,2,3,4-tetrahydroisoquinoline.

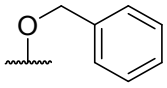
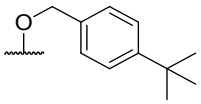
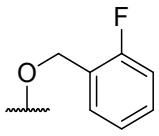
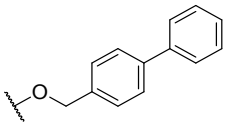
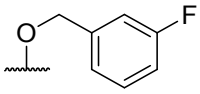
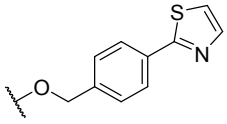
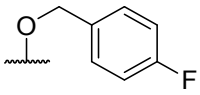
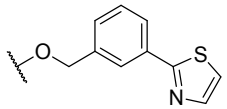
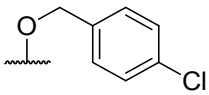
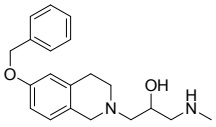
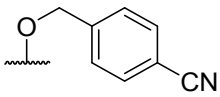
We then focused our attention on the benzyl ether scaffold represented by compound **21** and prepared a limited number of derivatives (Table 6). Introduction of the fluorine atom at 2-, 3-, 4-position (**38**-**40**) of the phenyl ring in compound **21** slightly impaired

the potency, indicating that substitution on the benzyl ether scaffold was more tolerated, respectively. Among the fluorine-substituted analogs, compound **40** with a fluorine atom at the *para*-position showed almost equal potency to **21**. Furthermore, we tried to install different groups at the *para*-position, such as chloride, cyano, tertiary butyl, phenyl and thiazolyl groups. The potency of compound **41** (IC_{50}= 0.037 μM) owning a chloride atom displayed significantly enhancement, which might profit from the introduction of potential Van der Waals (VDW) interactions between chloride atom and the surrounding residues (such as Tyr150 and Phe153). In contrast, incorporation of a tertiary butyl group at 4-position (**43**) resulted in a loss of potency. As expected, an enhanced potency was observed for the aromatic substituent derivatives (**44** and **45**), among which thiazolyl-substituted compound **45** (IC_{50} = 0.031μM) yielded the highest potency for PRMT4 in this series of compounds. Migration of thiazolyl group from the *para*-position to *meta*-position generated compound **46** (IC_{50} = 0.043μM) with slight loss of activity as compared to compound **45**, which was consistent with earlier SAR result. Similarly, 6-benzyl ether substituted derivatives (**47**) of 1,2,3,4-tetrahydroisoquinoline exhibited an IC_{50} value of 0.475 μM (3-fold worse than 5-substituted compound **21**).

Encouraged by the improvement of potency, chiral separation was performed to examine the effect of chirality on PRMT4 inhibitory activity. Eutomer **49** showed approximately 5-fold more potent than distomer **48**, suggesting that *R*-enantiomer was superior to the *S*-enantiomer. Ultimately, **49** was selected for further profiling because of its excellent potency for PRMT4.

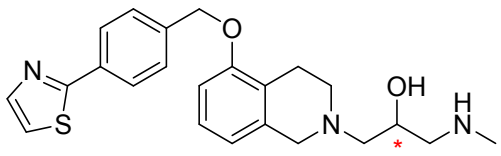
Table 6. PRMT4 enzymatic activity of compound 21, 36-47



Compd	R	$IC_{50}(\mu M)^a$	Compd	R	$IC_{50}(\mu M)^a$
21		0.165 ± 0.029	**43**		1.95 ± 0.34
38		0.254 ±0.011	**44**		0.120 ±0.002
39		0.530 ± 0.031	**45**		0.031 ± 0.010
40		0.228 ± 0.019	**46**		0.043 ± 0.006
41		0.037 ±0.004	**47**[b]		0.475 ± 0.037
42		0.305 ±0.042			

[a] All IC_{50} values are reported as the geometric mean from at least two determinations. [b] Benzyl ether substituted at 6-position of 1,2,3,4-tetrahydroisoquinoline.

Table 7. PRMT4 enzymatic activity of compound 45 and its enantiomers 48, 49



Compd	stereo[a]	$IC_{50}(\mu M)^b$

45	*rac*	0.031 ± 0.010
48	*S*	0.101 ±0.022
49	*R*	0.021 ± 0.005

[a] Chirality introduced from chiral starting material. [b] All IC_{50} values are reported as the geometric mean from at least two determinations

Selectivity profile of compound 49

We evaluated the selectivity of compound **49** against other PRMTs. As summarized in Table 8, compound **49** exhibited good selectivity for PRMT4 over other PRMTs (>100-fold). Specifically, the selectivity between PRMT4 and PRMT6, which shared the most structurally similarity with PRMT4 among the family, could reach up to 380-fold. To further assess the selectivity of compound **49**, we tested it against 7 common protein lysine methyltransferases (PKMTs) and delightly found that compound **49** did not significantly inhibit any of these PKMTs up to 50 μM (Figure 4).

Table 8. Selectivity evaluation of compound **49** [a]

Enzyme	Type I					Type II	Type III	Selectivity Ratio [b] (4vs.6)
	PRMT4	PRMT1	PRMT3	PRMT6	PRMT8	PRMT5	PRMT7	
IC_{50}(μM)[a]	0.021	8.6	>10	7.9	3.7	>30	>30	380

[a] All IC_{50} values are reported as the geometric mean from at least two determinations. [b] Selectivity ratio = IC_{50} of PRMT6/ IC_{50} of PRMT4

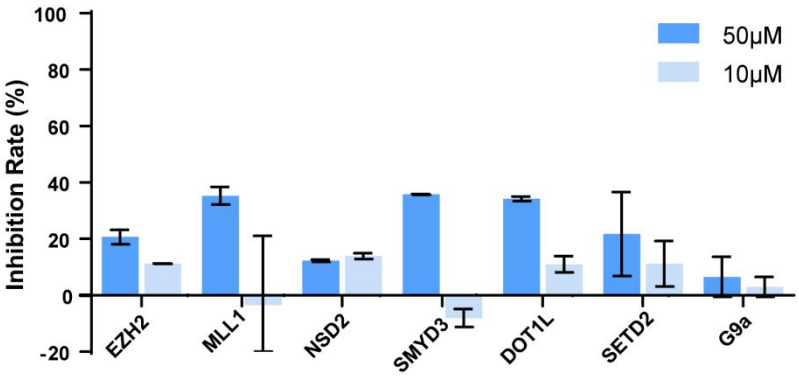


Figure 4. Inhibitory effect of compound **49** against PKMTs (n=2)

Compound 49 Inhibited Proliferation of Multiple Cancer Cell Lines

As known the essential role of PRMT4 for myeloid leukemogenesis [17], we tested 8 leukemia cell lines (MV4-11, MOLM13, THP-1, RS4.11, MOLT4, MOMOMAC6, HEL, K562) to detect whether compound **49** was sufficient to inhibit cancer cell proliferation (Table S2). Results showed that compound **49** was potent at inhibiting cell proliferation in a dose-dependent manner and notably showed high potency toward MOLM13 cell among leukemia cell lines with IC_{50} of 6.93 μM (Figure 5). Thus, we focused on MOLM13 cell for our further research.

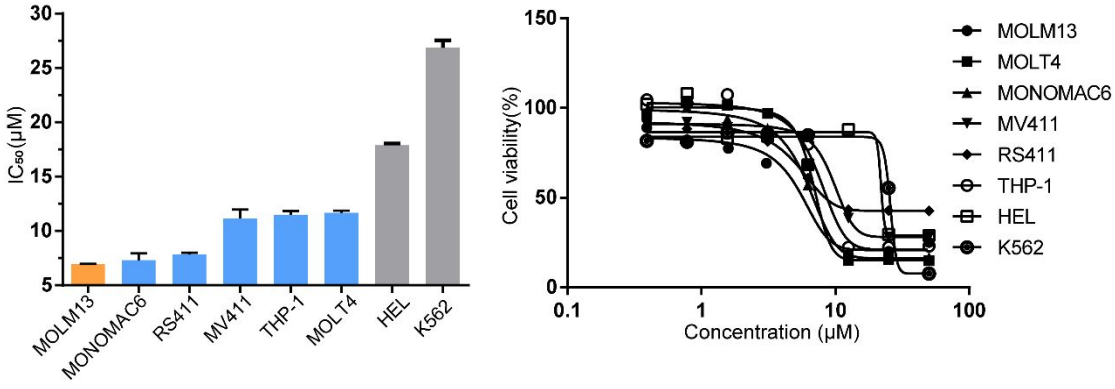


Figure 5. Proliferation inhibition of 8 leukemia cell lines by Compound **49** in vitro 6 days in culture, IC_{50} values were calculated in GraphPad Prism.

Compound 49 Decreased the Arginine Methylation at Cellular Levels

We chose MOLM13, the most sensitive cell line in the tested leukemia cells, to further assess the cellular function of compound **49** in vitro. After treatment with compound **49** for 96 h, we used specific antibodies of asymmetric dimethyl BAF155 and PABP1 to detect the asymmetric methylation and an aDMA antibody to assess the overall levels of asymmetric dimethyl arginine. Asymmetric dimethyl-BAF155 (IC_{50}=0.369 μM), PABP1 (IC_{50}=0.545 μM) and the global aDMA levels were reduced in a concentration-dependent manner (Figure6 and Figure S3A). We also evaluated the inhibitory effect of **49** on ectopically expressed PRMT4 in HEK293T cells. After treatment with compound **49**, the asymmetric dimethylation of BAF155, PABP1 and the level of aDMA in HEK293T cells with high expression of wild type PRMT4 (Figure S3B) were also decreased in a dose-dependent manner (Figure S3C), which was in line with the results in MOLM13 cells. These data together confirmed that **49** could inhibit the catalysis function of PRMT4 and resulted in reduction of asymmetric dimethylation of total aDMA, and two well-known substrate proteins BAF155 and PABP1 at cellular levels.

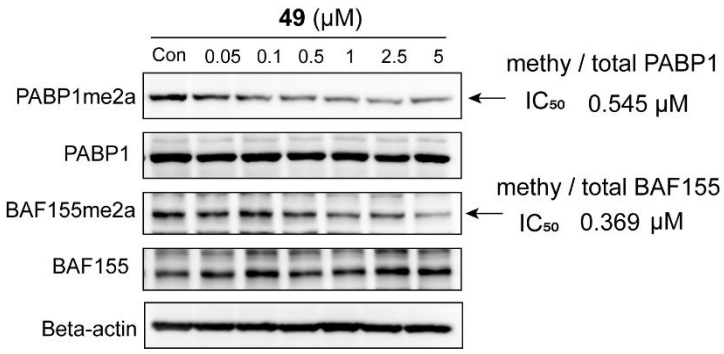


Figure 6. MOLM13 cells were treated with indicated concentration of compound **49** for 4 days and whole cell extracts were analyzed by western blotting for PABP1, BAF155 dimethylation. According to the grey scale of Image J, PABP1me2a and BAF155me2a signal intensities

normalized to total PABP1 and total BAF155, respectively. IC_{50} values were calculated in GraphPad Prism.

Although the roughly 10-fold difference between the antiproliferative activity and the cellular methylation inhibition is consistent with previous reported PRMT4 inhibitors, the slightly large gap between enzymatic activity and cellular methylation inhibition of **49** may stem from other factors rather than target relevant.[35, 36] By investigating the reported PRMT4 inhibitors, such as EZM2302 and TP-064, we found that different cell lines could affect the methylation inhibition activity and different molecules also harbor different physicochemical characteristics leading various cellular effects. Therefore, we thought that these two factors may account for the slightly large discrepancy between enzymatic activity and cellular methylation inhibition of **49**. Nevertheless, giving the similar antiproliferative activity of compound **49** with those of other PRMT4 inhibitors, we further evaluate its in vitro and vivo properties.

Compound 49 Induced G1 Cell Cycle Arrest and Apoptosis in MOLM13

To investigate the mechanism of antiproliferation in MOLM13 cells, we performed cell cycle analyses and apoptosis analyses by flow cytometry. Results showed that compound **49** decreased the proportion of MOLM13 cells in S phase, while increased the percentage of cells in G_0/G_1 phase in a dose-dependent manner (Figure 7A). As shown in Figure 7B, compound **49** also induced cell apoptosis in a concentration-dependent manner. Once MOLM13 cells were treated with compound **49** at 12 μM, the proportion of total apoptosis including the early stage apoptosis and the later stage of

apoptosis was 96.4%. These data indicated that the antiproliferative effect of compound **49** was dependent on arresting cell cycle and inducing apoptosis.

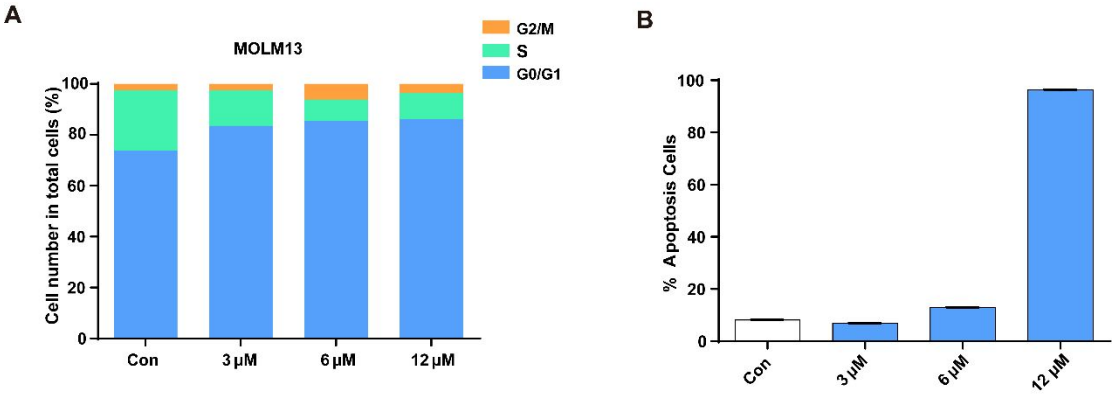


Figure 7. Cellular responses of MOLM13 cells treated with **49**. (A) Cell cycle of MOLM13 cells was arrested at G1 phase, after treatment with **49** from 3 to 12 μM for 72 h . (B) MOLM13 was induced apoptosis, after treatment with **49** from 3 to 12 μM for 96 h.

In vivo Pharmacokinetic Properties and Anti-tumor Effect in Xenografts

As compound **49** showed high potency in vitro, the pharmacokinetic (PK) profiles were next evaluated in ICR mice by intravenous and oral administration. A single dose of compound **49** at 3 mg/kg i.v. administration showed high volume of distribution at steady state (Vss) of 42.6 L/kg and moderate clearance (CL) of 44.9 mL/min/kg (the curve of drug concentrations in plasma was shown in Figure S2A). Besides, a slow oral absorption (T_{max} = 8.0 h), long half-life ($t_{1/2}$ = 24.1 h) and good oral bioavailability (F = 48.8%) at an oral dose of 10 mg/kg were observed. From detailed analysis of the plasma concentration at various time points, we could find that the inhibitor could retain about 120 nM concentration at most of time in the plasma after a single oral dosage of 10 mg/kg. We also determined the plasma protein binding of **49** (PPB, about 98%, Table S4), together with the high volume of distribution at steady state, indicating that

compound **49** had a capability to dissociate from plasma proteins and distributed into the tissue. By comparing the cellular activities, we presumed that a higher dosage is needed to offer sufficient drug concentration to exhibit antitumor efficacy. Therefore, we further studied the in vivo activity of compound **49**.

Table 9. Pharmacokinetic parameters for compound **49** in ICR (CD-1) mice [a]

Parameter	3 mg/kg i.v.	10 mg/kg p.o.
CL(mL/min/kg)	44.9	
V_{ss}(L/kg)	42.6	
$t_{1/2}$(h)	12.1	24.1
T_{max}(h)		8.0
C_{max}(ng/mL)		75.4
$AUC_{0\text{-}last}$(ng.h/mL)	869	1412
$AUC_{0\text{-}inf}$(ng.h/mL)	1165	3032
F (%)		48.8

[a] Mice (n = 3); Parameters were calculated from composite mean plasma concentration–time data. Compound dissolved in 1% Tween80/water.

To further evaluate the antitumor effect of compound **49**, we performed tumor growth inhibition studies in BALB/c nude mice bearing subcutaneous MOLM13 xenografts with daily oral dose of 100 mg/kg (Figure 8A-D). As demonstrated in Table 10, compound **49** showed potent therapeutic effect with tumor growth inhibition (TGI) rate of 53.5%. After sacrificing the xenograft mice, we analysized the intratumoral level of aDMA, BAF155me2a, and PABP1me2a, and the result showed a remarkably decrease

of asymmetrical dimethylation (Figure 8E), which was consistent with cellular results**.** We also tested the drug concentration in tumor tissues collected from in vivo pharmacological study. The result showed that the average of drug concentration in tumor tissues was about 80724 ng/g (Table S3). As compound **49** inhibited the proliferation of MOLM13 with an IC_{50} of 6.93 μM (equal to 2835 ng/mL), it meant the drug concentration in tumor tissues was about 20-30 folds higher than IC_{50} of the antiproliferative activities, which may explain the fact that compound **49** displayed the good anti-tumor activity in xenograft model.

Table 10. Summary of tumor growth inhibition of compound **49**

Tumor model	Compd	Administration		Survivors (day)	Tumor growth inhibition (%)
		Dose (mg/kg)	Route		
MOLM13	**49**	100	po	18	53.5(p<0.01)

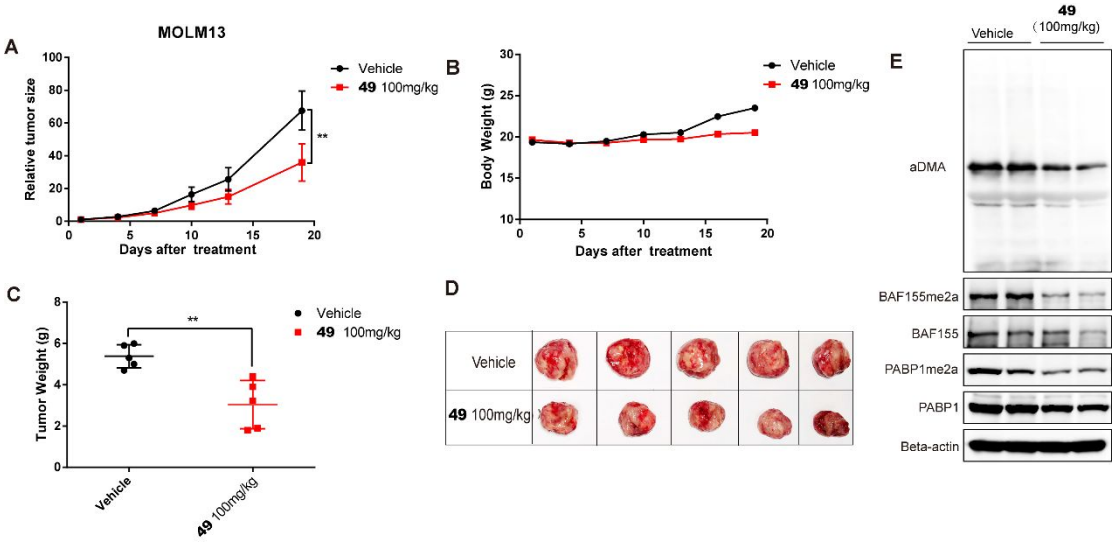
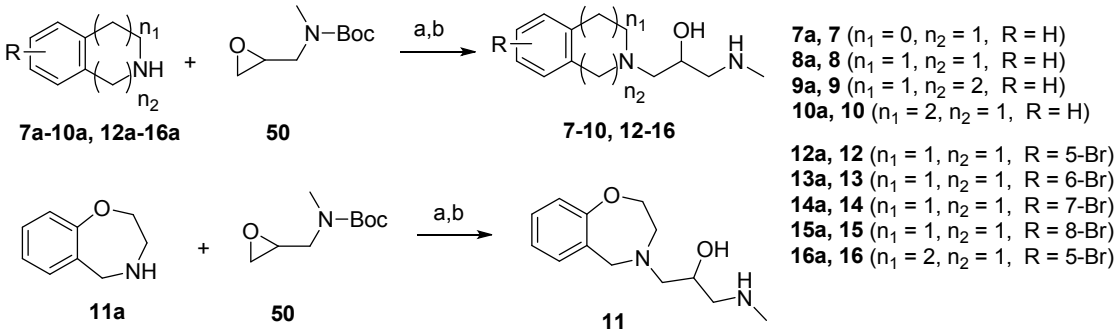


Figure 8. (A-E) Antitumor efficacy of **49** in vivo. Mice carrying MOLM13 xenograft model were treated with **49** in a dose of 100 mg/kg daily for indicated days. Error bars represent mean ± SD, n

≥5 mice per group for (A) and (C). (A) and (C) were analyzed by unpaired two tailed t test. **p < 0.01, ***p < 0.001. (B) Mean body weight of MOLM13 xengraft mice under the administration of compound **49** (data shown as mean values, n=5 mice per group). (D) Visual pictures showing representative tumor volumes of control and treatment group of MOLM13 model. (E) the asymmetric dimethylation of BAF155, PABP1 and the level of aDMA were detected by Western Blot. Beta-actin was used as loading controls.

Chemistry

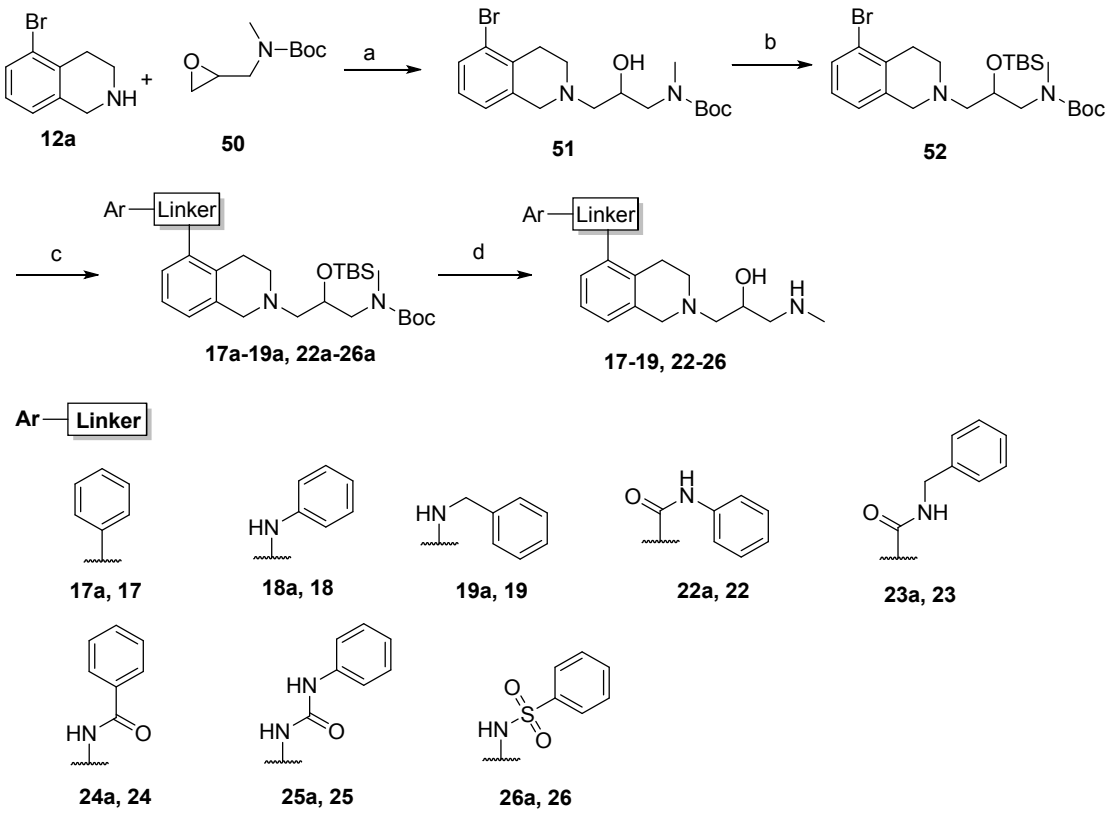
Scheme 1. Synthesis of compounds 7-16 [a]



[a] Reagents and conditions: (a) *i*-PrOH, 80 °C; (b) MeOH/HCl.

The preparation of compounds **7-16**, as depicted in Scheme 1, began with a nucleophilic substitution reaction between different commercially available cyclic secondary amine (**7a-16a**) and tert-butyl methyl(oxiran-2-ylmethyl) carbamate (**50**), followed by a deprotection reaction.

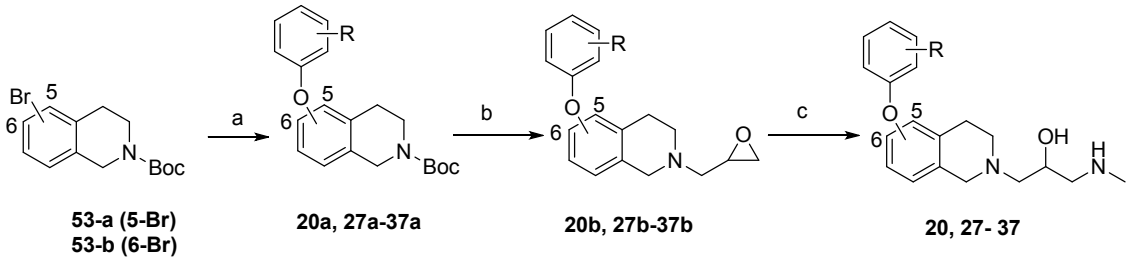
Scheme 2. Synthesis of compounds 17-19 and 22-26 [a]



[a] Reagents and conditions: (a) *i*-PrOH, 80 °C; (b) TBSCl, imidazole, DCM; (c) Coupling reaction or carbonyl extrusion condensation reaction; (d) MeOH/HCl.

Synthetic routes for preparing tetrahydroisoquinoline derivatives **17-19** and **22-26** with various linkers are outlined in Scheme 2. Intermediate **52** was prepared via a two-step sequence of reactions, involving a nucleophilic substitution reaction and addition of TBS protecting group. The resulting intermediate **52** was then subjected palladium or copper catalyzed coupling reaction or carbonyl extrusion condensation reaction and subsequent deprotection reaction to afford the corresponding end-product.

Scheme 3. Synthesis of phenyl ether derivatives 20 and 27-37 [a]

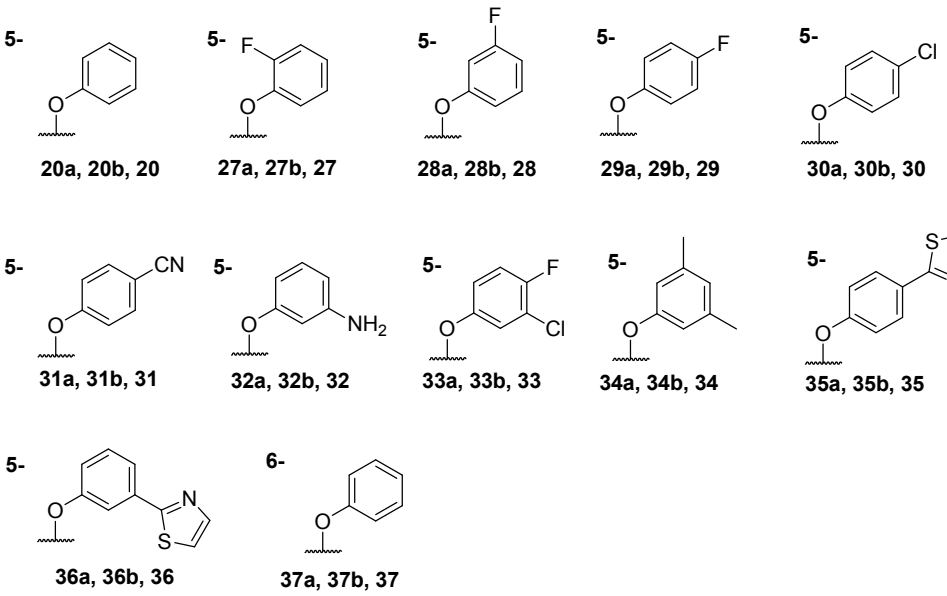


53-a (5-Br)
53-b (6-Br)

20a, 27a-37a

20b, 27b-37b

20, 27- 37



20a, 20b, 20 **27a, 27b, 27** **28a, 28b, 28** **29a, 29b, 29** **30a, 30b, 30**

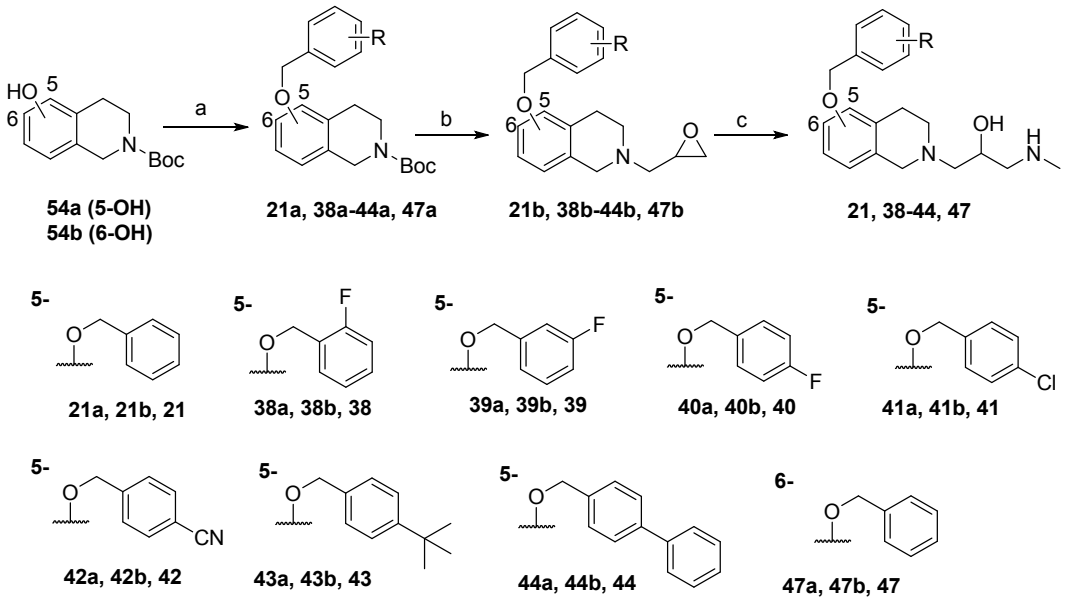
31a, 31b, 31 **32a, 32b, 32** **33a, 33b, 33** **34a, 34b, 34** **35a, 35b, 35**

36a, 36b, 36 **37a, 37b, 37**

[a] Reagents and conditions: (a) CuI, 2-picolinic acid, K_3PO_4, phenol, DMSO, 90 °C; (b) MeOH/HCl and then THF, epibromohydrin, KF, 45 °C; (c) MeOH/$MeNH_2$.

The synthesis of phenyl ether derivatives **20** and **27-37** is summarized in Scheme 3. A copper catalyzed Ullmann reaction between aryl bromide (**53a** or **53b**) and phenol afforded the intermediate **20a** and **27a-37a**. After removing the Boc protecting group and a nucleophilic substitution reaction, the intermediate **20b** and **27b-37b** were obtained. Sequential ethylene oxide ring opening reaction under the attack of methylamine produced the compounds **20** and **27-37**.

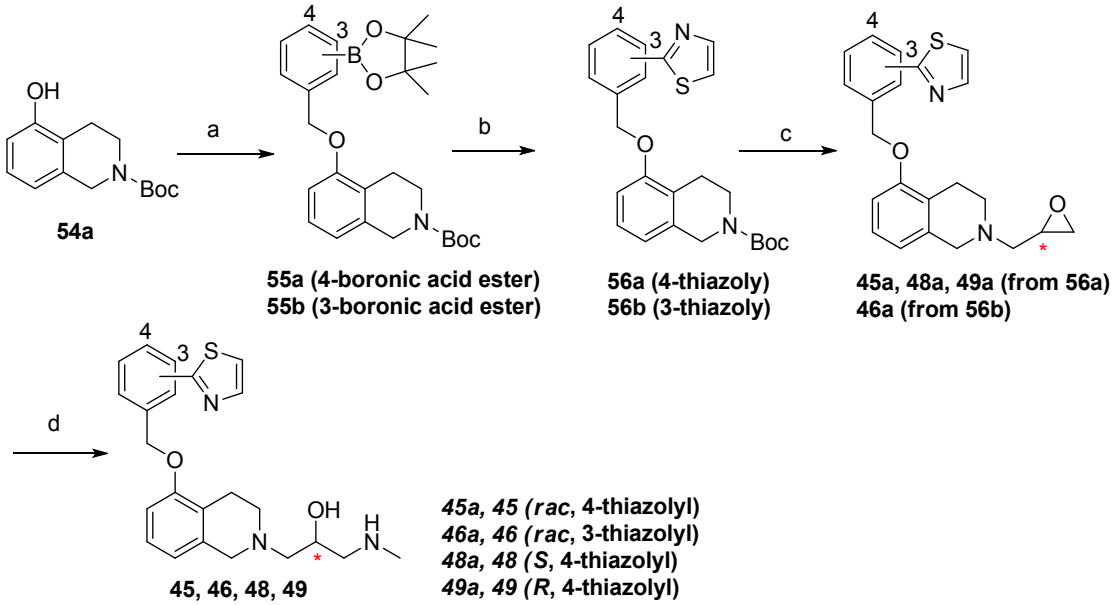
Scheme 4. Synthesis of benzyl ether derivatives 21, 38-44 and 47 [a]



[a] Reagents and conditions: (a) K_2CO_3, benzyl bromide derivatives, acetonitrile; (b) MeOH/HCl and then THF, epibromohydrin, KF, 45 °C; (c) MeOH/$MeNH_2$.

Besides, the benzyl ether derivatives **21, 38-44** and **47** were synthesized in a similar route to phenyl ether derivatives starting from a nucleophilic substitution reaction between Boc-protected 1,2,3,4-tetra-hydroisoquinolin-5-ol (**54a**) or 1,2,3,4-tetra-hydroisoquinolin-6-ol (**54b**) and the corresponding benzyl bromide derivatives.

Scheme 5. Synthesis of compound 45,46, 48 and 49 [a]



[a] Reagents and conditions: (a) K_2CO_3, benzyl bromide derivatives, acetonitrile； (b) Ph_3P, $Pd(Ph_3P)_4$, K_3PO_4, 2-bromothiazole, 1,4-dioxane/H_2O, 90 °C; (c) MeOH/HCl and then THF, epibromohydrin or S-glycidylnosylate or R-glycidylnosylate, KF, 45 °C; (d) MeOH/$MeNH_2$.

In consideration of the commercially unavailable of thiazolyl substituted benzyl bromide, compound **45**, **46**, **48** and **49** were synthesized in a slightly altered route. Boc-protected 1,2,3,4-tetrahydroisoquinolin-5-ol (**54a**) and boronic acid pinacol ester substituted benzyl bromide underwent a nucleophilic substitution reaction, followed by a Suzuki-coupling reaction with 2-bromothiazole to afford intermediate **56a** or **56b**. Then **56a** or **56b** subjected a deprotected reaction and another nucleophilic substitution reaction with epibromohydrin or S-glycidylnosylate or R-glycidylnosylate to yield the corresponding racemic (**45a** and **46a**) or enantiomeric (**48a** and **49a**) intermediate. Methylamine facilitated the SN_2 ring opening reaction to produce the end-product with chiral inversion.

CONCLUSIONS

Starting from a reported dual PRMT4/6 inhibitor **6**, we discovered a selective and in vivo effective PRMT4 inhibitor **49** through scaffold hopping strategy and subsequent structure-based optimization. Compound **49** exhibited high potency against PRMT4 (IC_{50} = 21 nM) and excellent selectivity over other PRMTs and PKMTs (>100-fold). Compound **49** could induce anti-proliferative effect on a panel of leukemia cancer cell lines by inducing cell cycle arrest at G1 phase and apoptosis. Oral administration of compound **49** demonstrated good pharmacokinetic profiles and significant anti-tumor activity in acute myeloid leukemia MOLM13 xenograft model, without the obvious

loss of body weight and visable toxicity. Importantly, reduction of the methylation of PRMT4 substrate proteins such as PABP1 and BAF155 were confirmed with in vivo pharmacodynamics study. Together, our findings may help to validate PRMT4 as a potential therapeutic anti-cancer target and provide a drug candidate for the treatment of acute myeloid leukemia.

EXPERIMENTAL SECTION

General Chemistry Information

^{1}H NMR (400 MHz) spectra were recorded by using a Varian Mercury-400 high performance digital FT-NMR spectrometer with tetramethylsilane (TMS) as an internal standard. ^{13}C NMR (100 or 125 MHz) spectra were recorded by using a Varian Mercury-400 high performance digital FT-NMR spectrometer or Varian Mercury-500 high performance digital FT-NMR spectrometer. NMR data are reported as follows: chemical shift, integration, multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; dd, doublet of doublets), and coupling constants. Very broad peaks for protons of, for example, hydroxyl and amino groups are not always indicated. Low-resolution mass spectra were obtained with a Finnigan LCQ Deca XP mass spectrometer using a CAPCELL PAK C18 (50 mm × 2.0 mm, 5 μm) or an Agilent ZORBAX Eclipse XDB C18 (50 mm × 2.1 mm, 5 μm) in positive or negative electrospray mode. High resolution mass spectra were recorded by using a Finnigan MAT-95 mass spectrometer or an Agilent technologies 6224 TOF mass spectrometer. The purity of compounds was determined by high-performance liquid chromatography (HPLC) and confirmed to be more than 95%. Purity of all compounds (except for compound **45** and **46**) was determined by analytical Gilson-215 high performance–liquid chromatography using an YMC ODS3 column (50 mm × 4.6 mm, 5 μm). Conditions were as follows: CH_3CN/H_2O eluent at 2.5 mL/min flow [containing 0.1% trifluoroacetic acid (TFA)] at 35 °C, 8 min, gradient 5% CH_3CN to 95% CH_3CN, monitored by UV absorption at 214 and 254 nm. Purity of compound **45** and **46** was determined by analytical Agilent-

1290 high performance–liquid chromatography using a Waters BEH C18 column (50 mm × 2.1 mm, 1.7 μm). Conditions were as follows: CH_3CN/H_2O eluent at 0.5 mL/min flow [containing 0.1% trifluoroacetic acid (TFA)] at 40 °C, 5 min, gradient 5% CH_3CN to 80% CH_3CN, monitored by UV absorption at 214 and 254 nm. TLC analysis was carried out with glass precoated silica gel GF254 plates. TLC spots were visualized under UV light. All solvents and reagents were used directly as obtained commercially unless otherwise noted. All air and moisture sensitive reactions were carried out under an atmosphere of dry argon with heat-dried glassware and standard syringe techniques.

1-(isoindolin-2-yl)-3-(methylamino)propan-2-ol (**7**). The title compound was obtained as a colorless oil using a method similar to that described for compound **8** in 27% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.19 (m, 4H), 4.12 – 4.05 (m, 2H), 3.99 – 3.94 (m, 2H), 3.93 – 3.88 (m, 1H), 2.87 (dd, *J* = 12.0, 9.4 Hz, 1H), 2.80-2.65 (brs, 2H), 2.73 (dd, *J* = 12.0, 3.6 Hz, 3H), 2.62 (dd, *J* = 12.0, 7.5 Hz, 1H), 2.48 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 139.8, 126.9, 122.3, 67.4, 60.0, 59.4, 55.6, 36.2. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{12}H_{19}N_2O$ 207.1492, found 207.1496. Retention time 1.37 min, HPLC purity = 100%.

1-(3,4-dihydroisoquinolin-2(1H)-yl)-3-(methylamino)propan-2-ol (**8**). A suspension of 1,2,3,4-tetrahydro-isoquinolin **8a** (160 mg, 1.2 mmol) in *i*-PrOH (5 mL) was added *tert*-butyl methyl(oxiran-2-ylmethyl) carbamate **50** (270 mg, 1.44 mmol). The reaction solution was heated at 80 °C overnight before being concentrated and purified by column chromatography on silica gel to afford the intermediate. Boc protecting group was removed using general procedure (HCl/MeOH) to yield the end-

product as colorless oil (94 mg, 36% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 – 7.06 (m, 3H), 7.04 – 6.97 (m, 1H), 4.02 – 3.90 (m, 1H), 3.78 (d, *J* = 14.9 Hz, 1H), 3.60 (d, *J* = 14.9 Hz, 1H), 2.96-2.83 (m, 5H), 2.77 – 2.69 (m, 1H), 2.66 (dd, *J* = 12.0, 3.4 Hz, 1H), 2.62 – 2.54 (m, 2H), 2.48 (dd, *J* = 12.4, 3.6 Hz, 1H), 2.44 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.4, 134.1, 128.7, 126.6, 126.3, 125.7, 65.8, 61.9, 56.2, 55.6, 51.2, 36.2, 29.0. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{13}H_{21}N_2O$ 211.1648, found 211.1648. Retention time 1.37 min, HPLC purity >99%.

1-(4,5-dihydro-1H-benzo[d]azepin-3(2H)-yl)-3-(methylamino)propan-2-ol (**9**). The title compound was obtained as a colorless oil using a method similar to that described for compound **8** in 54% yield over two steps. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 – 7.05 (m, 4H), 3.94 – 3.81 (m, 1H), 2.98 – 2.88 (m, 4H), 2.88 – 2.76 (m, 2H), 2.70 – 2.57 (m, 4H), 2.57 – 2.53 (m, 1H), 2.50 (dd, *J* = 6.7, 2.7 Hz, 2H), 2.46 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 141.8, 129.0, 126.3, 65.8, 62.0, 55.8, 55.7, 36.62, 36.60. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{13}H_{23}N_2O$ 235.1805, found 235.1804. Retention time 1.82 min, HPLC purity = 100%.

1-(4,5-dihydro-1H-benzo[c]azepin-2(3H)-yl)-3-(methylamino)propan-2-ol (**10**). The title compound was obtained as a colorless oil using a method similar to that described for compound **8** in 39% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 – 7.06 (m, 4H), 3.91 (s, 2H), 3.87 – 3.77 (m, 1H), 3.26 – 3.05 (m, 2H), 2.90 (dd, *J* = 10.0, 3.8 Hz, 2H), 2.67 – 2.52 (brs, 2H), 2.55 (dd, *J* = 12.3, 3.4 Hz, 1H), 2.47 (dd, *J* = 12.3, 7.6 Hz, 1H), 2.47 – 2.39 (m, 1H), 2.41 (s, 3H), 2.32 – 2.18 (m, 1H), 1.75 – 1.64 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 142.8, 138.7, 129.9, 129.0, 127.4, 126.0, 65.6,

59.5, 59.3, 56.1, 55.7, 36.3, 36.1, 25.3. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{13}H_{23}N_2O$ 235.1805, found 235.1809. Retention time 1.63 min, HPLC purity > 95%.

1-(2,3-dihydrobenzo[f][1,4]oxazepin-4(5H)-yl)-3-(methylamino)propan-2-ol (**11**). The title compound was obtained as a colorless oil using a method similar to that described for compound **8** in 56% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 – 7.15 (m, 1H), 7.10 (dd, *J* = 7.3, 1.5 Hz, 1H), 7.03 – 6.95 (m, 2H), 4.10 – 3.96 (m, 2H), 3.88 (q, *J* = 12.3 Hz, 2H), 3.83 (td, *J* = 7.2, 3.5 Hz, 1H), 3.13 (ddd, *J* = 14.9, 6.2, 2.5 Hz, 2H), 2.60 (dd, *J* = 12.0, 3.5 Hz, 1H), 2.52 (ddd, *J* = 19.2, 12.3, 5.3 Hz, 2H), 2.41 (s, 3H), 2.37 (dd, *J* = 12.3, 9.9 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.8, 131.3, 130.7, 128.7, 123.5, 120.9, 70.0, 66.1, 58.6, 58.2, 57.2, 55.6, 36.3. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{13}H_{21}N_2O_2$ 237.1598, found 237.1592. Retention time 1.36 min, HPLC purity > 99%.

1-(5-bromo-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methylamino)propan-2-ol (**12**). The title compound was obtained as a colorless oil using a method similar to that described for compound **8** in 57% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.04 – 6.94 (m, 2H), 3.99 – 3.91 (m, 1H), 3.78 (d, *J* = 15.0 Hz, 1H), 3.61 (d, *J* = 15.0 Hz, 1H), 2.97-2.89 (m , 1H), 2.85 (td, *J* = 5.4, 1.2 Hz, 2H), 2.79 – 2.72 (m, 1H), 2.68 (dd, *J* = 12.0, 3.5 Hz, 1H), 2.64 – 2.54 (m, 2H), 2.49 (dd, *J* = 12.5, 3.5 Hz, 1H), 2.45 (s, 3H), 2.31 (brs, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 137.0, 134.0, 130.3, 127.1, 125.7, 125.2, 66.1, 61.5, 56.2, 55.7, 51.2, 36.5, 30.1. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{13}H_{20}BrN_2O$ 299.0754, found 299.0760. Retention time 1.89 min, HPLC purity >99%.

1-(6-bromo-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methylamino)propan-2-ol (**13**). The title compound was obtained as a colorless oil using a method similar to that described for compound **8** in 44% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 – 7.20 (m, 2H), 6.88 (d, *J* = 8.0 Hz, 1H), 4.00 – 3.90 (m, 1H), 3.72 (d, *J* = 15.1 Hz, 1H), 3.56 (d, *J* = 15.1 Hz, 1H), 2.92 – 2.83 (m, 3H), 2.70 – 2.65 (m, 2H), 2.63 – 2.53 (m, 2H), 2.49 (dd, *J* = 12.5, 3.6 Hz, 1H), 2.45 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 136.5, 133.5, 131.4, 128.8, 128.2, 119.8, 66.0, 61.8, 55.7, 50.8, 36.4, 28.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{13}H_{20}BrN_2O$ 299.0754, found 299.0756. Retention time 1.98 min, HPLC purity = 100%.

1-(7-bromo-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methylamino)propan-2-ol (**14**). The title compound was obtained as a colorless oil using a method similar to that described for compound **8** in 30% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.15 (d, *J* = 2.1 Hz, 1H), 6.96 (d, *J* = 8.2 Hz, 1H), 3.99 – 3.88 (m, 1H), 3.74 (d, *J* = 15.2 Hz, 1H), 3.58 (d, *J* = 15.2 Hz, 1H), 2.93-2.86 (m, 1H), 2.82 (t, *J* = 4.2 Hz, 2H), 2.74 – 2.68 (m, 2H), 2.66 (d, *J* = 3.5 Hz, 1H), 2.62 – 2.53 (m, 3H), 2.49 (dd, *J* = 12.5, 3.5 Hz, 2H), 2.45 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 136.7, 133.1, 130.4, 129.4, 119.2, 65.9, 61.6, 55.7, 55.6, 50.9, 36.2, 28.5. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{13}H_{20}BrN_2O$ 299.0754, found 299.0755. Retention time 1.96 min, HPLC purity > 99%.

1-(8-bromo-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methylamino)propan-2-ol (**15**). The title compound was obtained as a colorless oil using a method similar to that described for compound **8** in 32% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ

7.36 (d, *J* = 7.5 Hz, 1H), 7.09 – 6.98 (m, 2H),4.02-3.96 (m, 1H), 3.76 (d, *J* = 15.9 Hz, 1H), 3.60 (d, *J* = 15.9 Hz, 1H), 2.95 – 2.84 (m, 3H), 2.75 – 2.70 (m, 1H), 2.70 – 2.59 (m, 3H), 2.56 (dd, *J* = 12.4, 3.5 Hz, 1H), 2.47 (s, 3H), 2.23 (brs, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 137.2, 134.0, 130.0, 127.9, 127.5, 122.9, 66.0, 61.8, 56.9, 55.7, 50.4, 36.5, 29.7. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{13}H_{20}BrN_2O$ 299.0754, found 299.0756. Retention time 1.81 min, HPLC purity = 100%.

1-(6-bromo-4,5-dihydro-1H-benzo[c]azepin-2(3H)-yl)-3-(methylamino)propan-2-ol (**16**). The title compound was obtained as a colorless oil using a method similar to that described for compound **8** in 21% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.02 (dd, *J* = 6.5, 0.8 Hz, 1H), 6.96 – 6.90 (m, 1H), 3.93 (s, 2H), 3.86 – 3.75 (m, 1H), 3.25 – 3.05 (m, 4H), 2.55 (dd, *J* = 11.9, 3.4 Hz, 2H), 2.47 (dd, *J* = 11.9, 7.2 Hz, 1H), 2.41 (s, 3H), 2.43 – 2.38 (m, 1H), 2.25 (dd, *J* = 12.7, 10.1 Hz, 1H), 1.72 – 1.64 (m, *J* = 9.6, 4.8 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 141.9, 141.0, 132.0, 129.3, 127.2, 124.6, 65.8, 59.5, 59.1, 56.5, 55.7, 36.4, 33.8, 24.4. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{14}H_{22}BrN_2O$ 313.0910, found 313.0906. Retention time 2.03 min, HPLC purity > 98%.

1-(methylamino)-3-(5-phenyl-3,4-dihydroisoquinolin-2(1H)-yl)propan-2-ol (**17**). A suspension of *tert*-butyl(3-(5-bromo-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-((*tert*-butyl-dimethylsilyl)oxy)propyl)(methyl)carbamate **52** (128 mg, 0.25 mmol), phenyl-boronic acid (60 mg, 0.5 mmol) and Na_2CO_3 (53 mg, 0.5 mmol) in dioxane and water (3:1, 8 mL) was treated with $Pd(Ph_3P)_4$ (29 mg, 0.025 mmol). The system was purged with argon three times, then heated at 80 °C for 4 h, cooled down to room temperature,

filtered through silica gel and washed with EA. The combined filtrated was concentrated and resulting residue was purified by column chromatography on silica gel to afford the intermediate. Boc protecting group was removed using general procedure (HCl/MeOH) to yield the end-product as colorless oil (41 mg, 55% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.37 (m, 2H), 7.36 – 7.28 (m, 3H), 7.21 (t, *J* = 7.5 Hz, 1H), 7.10 (d, *J* = 7.5 Hz, 1H), 7.04 (d, *J* = 7.5 Hz, 1H), 4.02 – 3.93 (m, 1H), 3.89 (d, *J* = 15.0 Hz, 1H), 3.71 (d, *J* = 15.0 Hz, 1H), 2.88-2.80 (m, 1H), 2.76 (t, *J* = 5.6 Hz, 2H), 2.70 (dd, *J* = 12.0, 3.5 Hz, 1H), 2.67 – 2.56 (m, 4H), 2.50 (dd, *J* = 12.4, 3.5 Hz, 1H), 2.47 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 142.0, 141.3, 134.8, 131.9, 129.1, 128.1, 127.8, 127.0, 125.8, 125.7, 66.1, 61.9, 56.7, 55.8, 51.4, 36.6, 28.5. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{25}N_2O$ 297.1961, found 297.1966. Retention time 2.29 min, HPLC purity > 99%.

1-(methylamino)-3-(5-(phenylamino)-3,4-dihydroisoquinolin-2(1H)-yl)propan-2-ol (**18**). A suspension of *tert*-butyl(3-(5-bromo-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-((*tert*-butyldimethylsilyl)oxy)propyl)(methyl)carbamate **52** (205 mg, 0.40 mmol) in 6mL of toluene was added aniline (56 mg, 0.60 mmol), $Pd_2(dba)_3$ (21 mg, 0.024 mmol), XPhos (34 mg, 0.072 mmol), and Cs_2CO_3 (260 mg, 0.80 mmol). The system was purged with argon three times, then heated at 80 °C for 12 h, cooled down to room temperature, filtered through silica gel and washed with EA. The combined filtrated was concentrated and resulting residue was purified by column chromatography on silica gel to afford the intermediate. Boc protecting group was removed using general procedure (HCl/MeOH) to yield the end-product as colorless oil (60 mg, 48% over two

steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.22 (m, 2H), 7.16 – 7.05 (m, 2H), 6.98 (dd, *J* = 8.5, 0.9 Hz, 2H), 6.92 (t, *J* = 7.3 Hz, 1H), 6.70 (d, *J* = 7.0 Hz, 1H), 5.36 (s, 1H), 4.03 – 3.93 (m, 1H), 3.82 (d, *J* = 14.9 Hz, 1H), 3.63 (d, *J* = 14.9 Hz, 1H), 3.02 – 2.89 (m, 1H), 2.82-2.75 (m, 1H), 2.74 – 2.67 (m, 3H), 2.64 – 2.56 (m, 3H), 2.51 (dd, *J* = 12.4, 3.4 Hz, 2H), 2.46 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.6, 141.1, 135.8, 129.3, 126.3, 124.4, 120.8, 120.1, 118.0, 116.1, 66.0, 61.8, 56.4, 55.7, 51.0 , 36.4 , 25.0. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{26}N_3O$ 312.2070, found 312.2071. Retention time 2.31 min, HPLC purity > 95%.

1-(5-(benzylamino)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methylamino)propan-2-ol (**19**). The title compound was obtained as a colorless oil using a method similar to that described for compound **18** in 60% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.33 (m, 4H), 731 – 7.26 (m, 1H), 7.05 (t, *J* = 7.8 Hz, 1H), 6.48 (t, *J* = 7.3 Hz, 2H), 4.37 (d, *J* = 5.3 Hz, 2H), 4.02 – 3.95 (m, 1H), 3.86 (t, *J* = 5.2 Hz, 1H), 3.79 (d, *J* = 14.8 Hz, 1H), 3.60 (d, *J* = 14.8 Hz, 1H), 3.03 – 2.95 (m, 1H), 2.85 – 2.75 (m, 1H), 2.70 (dd, *J* = 12.0, 3.5 Hz, 2H), 2.66 – 2.54 (m, 5H), 2.50 (dd, *J* = 12.4, 3.5 Hz, 1H), 2.47 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 145.5, 139.3, 134.8, 128.7, 127.5, 127.3, 126.7, 118.4, 115.7, 108.0, 65.5, 61.5, 56.6, 55.3, 51.0, 48.2, 35.7, 24.2. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{28}N_3O$ 326.2227, found 326.2224. Retention time 2.36 min, HPLC purity > 97%.

Tert-butyl 5-phenoxy-3,4-dihydroisoquinoline-2(1H)-carboxylate (**20a**). A suspension of *tert*-butyl 5-bromo-3,4-dihydroisoquinoline-2(1*H*)-carboxylate **53a** (312 mg, 1.0 mmol) in 6 mL DMSO was added phenol (141 mg, 1.5 mmol), CuI (19 mg,

0.10 mmol), K_3PO_4 (424 mg, 2.0 mmol), and picolinic acid (25 mg, 0.20 mmol). The system was purged with argon three times, then heated at 90 °C for 12 h, cooled down to room temperature. The mixture was added with water and extracted with EA for three times. Combined organic phases were washed with saturated NaCl, then dried with anhydrous Na_2SO_4, filtered and concentrated. The resulting residue was purified by column chromatography on silica gel to afford the product as white solid (310 mg, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (t, J = 7.8 Hz, 2H), 7.15 (t, J = 8.1 Hz, 1H), 7.07 (t, J = 7.4 Hz, 1H), 6.95 – 6.90 (m, 3H), 6.78 (d, J = 8.1 Hz, 1H), 4.62 (s, 2H), 3.64 (s, 2H), 2.78 (s, 2H), 1.50 (s, 9H).

2-(oxiran-2-ylmethyl)-5-phenoxy-1,2,3,4-tetrahydroisoquinoline (**20b**). A suspension *tert*-butyl 5-phenoxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate **20a** (135 mg, 0.60 mmol) in MeOH (4 mL) was added HCl/dioxane (4 M solution, 1 mL). The solution was stirred at room temperature for 2 h prior to removal of all solvents under reduced pressure. The residue was added saturated $NaHCO_3$ aqueous solution and extracted with DCM. The organic was dried with anhydrous Na_2SO_4 and removed all solvents under reduced pressure to afford the intermediate as white solid without further purification. The intermediate was dissolved in THF (6 mL) and then added $KF{\cdot}2H_2O$ (85 mg, 0.90 mmol) and 2-(bromomethyl)oxirane (122 mg, 0.90 mmol). The result solution was stirred at 45 °C overnight, filtered and concentrated and resulting residue was purified by column chromatography on silica gel to afford the product as colorless oil (112 mg, 66% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (t, *J* = 7.3 Hz, 2H), 7.11 (t, *J* = 7.8 Hz, 1H), 7.05 (t, *J* = 7.2 Hz, 1H), 6.93 (d, *J* = 8.0 Hz, 2H), 6.87 (d,

J = 7.6 Hz, 1H), 6.75 (d, *J* = 8.0 Hz, 1H), 3.85 (d, *J* = 15.0 Hz, 1H), 3.71 (d, *J* = 15.0 Hz, 1H), 3.24 – 3.17 (m, 1H), 2.96 (d, *J* = 13.2 Hz, 1H), 2.89 – 2.74 (m, 5H), 2.58 – 2.51 (m, 1H), 2.44 (dd, *J* = 13.2, 6.8 Hz, 1H). MS (ESI) m/z 282.2 $[M+H]^+$.

1-(methylamino)-3-(5-phenoxy-3,4-dihydroisoquinolin-2(1H)-yl)propan-2-ol (**20**). A suspension *tert*-butyl 2-(oxiran-2-ylmethyl)-5-phenoxy-1,2,3,4-tetra- hydro-isoquinoline (112 mg, 0.40 mmol) in MeOH (4 mL) was slowly added a 30% solution of $MeNH_2$ in MeOH (0.5 mL). The result solution was stirred at room temperature for 12 h, concentrated and resulting residue was purified by column chromatography on silica gel to afford the product as colorless oil (84 mg, 67%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.27 (m, 2H), 7.10 (t, *J* = 7.9 Hz, 1H), 7.06 (t, *J* = 7.9 Hz, 1H), 6.96 – 6.89 (m, 2H), 6.83 (d, *J* = 7.6 Hz, 1H), 6.74 (d, *J* = 7.9 Hz, 1H), 4.00-3.91 (m, 1H), 3.83 (d, *J* = 15.1 Hz, 1H), 3.65 (d, *J* = 15.0 Hz, 1H), 2.96 – 2.86(m, 1H), 2.86 – 2.77 (m, 2H), 2.75 – 2.67 (m, 2H), 2.66 – 2.55 (m, 3H), 2.51 (dd, *J* = 12.4, 3.4 Hz, 2H), 2.46 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.6, 154.4, 136.8, 129.8, 126.7, 126.4, 122.8, 122.0, 117.8, 116.9, 66.0, 61.9, 56.1, 55.7, 50.9, 50.6, 36.4, 24.0. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{25}N_2O_2$ 313.1911, found 313.1916. Retention time 2.34 min, HPLC purity > 96%.

Tert-butyl 5-(benzyloxy)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**21a**). A suspension *tert*-butyl 5-hydroxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate **54a** (100 mg, 0.40 mmol) in acetonitrile (6 mL) was added Cs_2CO_3(191 mg, 0.59 mmol) and benzyl bromide (102 mg, 0.60 mmol). The result solution was stirred at room temperature for 2h, filtered and concentrated and resulting residue was purified by

column chromatography on silica gel to afford the product as colorless oil (108 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.42 (m, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.34 (d, *J* = 7.1 Hz, 1H), 7.14 (t, *J* = 7.9 Hz, 1H), 6.76 (t, *J* = 7.9 Hz, 2H), 5.08 (s, 2H), 4.58 (s, 2H), 3.66 (t, *J* = 4.2 Hz, 2H), 2.84 (t, *J* = 4.2 Hz, 2H), 1.50 (s, 9H).

5-(benzyloxy)-2-(oxiran-2-ylmethyl)-1,2,3,4-tetrahydroisoquinoline (**21b**). A suspension *tert*-butyl 5-(benzyloxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate **21a** (108 mg, 0.31 mmol) in MeOH (4 mL) was added HCl/dioxane (4 M solution, 1 mL). The solution was stirred at room temperature for 2 h prior to removal of all solvents under reduced pressure. The residue was added saturated $NaHCO_3$ aqueous solution and extracted with DCM. The organic was dried with anhydrous Na_2SO_4 and removed all solvents under reduced pressure to afford the intermediate as white solid without further purification. The intermediate was dissolved in THF (5 mL) and then added $KF{\cdot}2H_2O$ (44 mg, 0.47 mmol) and 2-(bromomethyl)oxirane (64 mg, 0.47 mmol). The result solution was stirred at room temperature overnight, filtered and concentrated and resulting residue was purified by column chromatography on silica gel to afford the product as colorless oil (80 mg, 87% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.41 (m, 2H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 1H), 7.09 (t, *J* = 8.1 Hz, 1H), 6.73 (d, *J* = 8.1 Hz, 1H), 6.69 (d, *J* = 7.7 Hz, 1H), 5.08 (s, 2H), 3.80 (d, *J* = 14.9 Hz, 1H), 3.68 (d, *J* = 14.9 Hz, 1H), 3.22 (td, *J* = 6.6, 3.3 Hz, 1H), 2.98 – 2.77 (m, 6H), 2.56 (dd, *J* = 4.9, 2.7 Hz, 1H), 2.46 (dd, *J* = 13.3, 6.7 Hz, 1H). MS (ESI) m/z 296.2 $[M+H]^+$.

1-(5-(benzyloxy)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methylamino)propan-2-ol (**21**). A suspension 5-(benzyloxy)-2-(oxiran-2-ylmethyl)-1,2,3,4- tetrahydro-isoquinoline **21b** (80 mg, 0.27 mmol) in MeOH (3 mL) was slowly added a 30% solution of $MeNH_2$ in MeOH (0.5 mL). The result solution was stirred at room temperature for 12 h, concentrated and resulting residue was purified by column chromatography on silica gel to afford the product as colorless oil (40 mg, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.35 (m, 4H), 7.34 – 7.28 (m, 1H), 7.07 (t, *J* = 7.9 Hz, 1H), 6.72 (d, *J* = 8.1 Hz, 1H), 6.65 (d, *J* = 7.9 Hz, 1H), 5.05 (s, 2H), 4.28 – 4.15 (m, 1H), 3.72 (d, *J* = 14.9 Hz, 1H), 3.62 (d, *J* = 14.9Hz, 1H), 2.94 (dd, *J* = 12.2, 2.9 Hz, 1H), 2.90 – 2.72 (m, 5H), 2.65 – 2.59 (m, 1H), 2.58 (s, 3H), 2.51 (dd, *J* = 12.2, 4.9 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.3, 137.3, 135.8, 128.5, 127.8, 127.1, 126.4, 123.4, 119.0, 108.9, 69.7, 65.1, 61.6, 56.3, 54.9, 51.2, 35.1, 23.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{27}N_2O_2$ 327.2067, found 327.2059. Retention time 2.40 min, HPLC purity > 97%.

2-(2-hydroxy-3-(methylamino)propyl)-N-phenyl-1,2,3,4-tetrahydroisoquinoline -5-carboxamide (**22**). A suspension of *tert*-butyl (3-(5-bromo-3,4-dihydroisoquinolin -2(1*H*)-yl)-2-((*tert*-butyldimethylsilyl)oxy)propyl)(methyl)carbamate **52** (160 mg, 0.40 mmol) in 1,4-dioxane (6 mL) was added Pd(dppf)Cl_2 (32 mg, 0.04 mol), Et_3N (81 mg, 0.80 mmol), aniline (74 mg, 0.80 mmol), DMAP (49 mg, 0.40 mmol) and flushed with argon for 5 min. Then DBU (91 mg, 0.60 mmol) and $Mo(CO)_6$ (53mg, 0.20 mmol) was added to the solution. The sealed system was heated at 85 °C for 16 h, cooled down to room

temperature, filtered through silica gel and washed with EA. The combined filtrated was concentrated and resulting residue was purified by column chromatography on silica gel to afford the intermediate. Boc protecting group was removed using general procedure (HCl/MeOH) to yield the end-product as colorless oil (20 mg, 15% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 7.61 (d, *J* = 7.9 Hz, 2H), 7.32 (t, *J* = 7.9 Hz, 3H), 7.17 (t, *J* = 7.6 Hz, 1H), 7.14 – 7.05 (m, 2H), 4.07 (s, 1H), 3.70 (q, *J* = 15.2 Hz, 2H), 3.10 – 3.00 (m, 2H), 2.95 – 2.84 (m, 1H), 2.76 (d, *J* = 10.0 Hz, 1H), 2.64 – 2.55 (m, 3H), 2.42 (s, 3H). 2.41 – 2.38 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.0, 138.3, 137.0, 135.6, 131.9, 129.1, 128.5, 125.9, 124.6, 124.4, 119.7, 65.8, 62.6, 56.9, 55.6, 50.8, 36.2, 27.1. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{26}N_3O_2$ 340.2020, found 340.2024. Retention time 2.09 min, HPLC purity = 100%.

N-benzyl-2-(2-hydroxy-3-(methylamino)propyl)-1,2,3,4-tetrahydro-isoquinoline-5-carboxamide (**23**). The title compound was obtained as a colorless oil using a method similar to that described for compound **22** in 37% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (s, 1H), 7.36 – 7.31 (m, 4H), 7.30-7.25 (m, 1H), 7.21 (d, *J* = 6.9 Hz, 1H), 7.12 (t, *J* = 7.5 Hz, 1H), 7.03 (d, *J* = 7.5 Hz, 1H), 4.67 (dd, *J* = 14.5, 6.1 Hz, 1H), 4.46 (dd, *J* = 14.4, 5.0 Hz, 1H), 3.76 – 3.65 (m, 1H), 3.63 (d, *J* = 4.5 Hz, 2H), 3.10 – 2.92 (m, 3H), 2.89 – 2.80 (m, 1H), 2.53 – 2.44 (m, 2H), 2.31 – 2.27 (m, 2H), 2.23 (dd, *J* = 12.6, 2.4 Hz, 1H), 2.20 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.7, 138.3, 136.7, 135.4, 131.8, 128.8, 128.1, 128.0, 127.6, 125.7, 124.6, 65.8, 62.5, 56.9, 55.4, 50.8, 43.9, 36.2, 27.0 HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{28}N_3O_2$ 354.2176, found 354.2181. Retention time 2.08 min, HPLC purity > 98%.

N-(2-(2-hydroxy-3-(methylamino)propyl)-1,2,3,4-tetrahydroisoquinolin-5-yl)benzamide (**24**). A suspension of *tert*-butyl(3-(5-bromo-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-((*tert*-butyldimethylsilyl)oxy)propyl)(methyl)carbamate **52** (154 mg, 0.30 mmol) in 5 mL of dioxane was added benzamide (55 mg, 0.45 mmol), $Pd(OAc)_2$ (7 mg, 0.03 mmol), XantPhos (26 mg, 0.045 mmol), and Cs_2CO_3 (145 mg, 0.45 mmol). The system was purged with argon three times, then heated at 80 °C for 12 h, cooled down to room temperature, filtered through silica gel and washed with EA. The combined filtrated was concentrated and resulting residue was purified by column chromatography on silica gel to afford the intermediate. Boc protecting group was removed using general procedure (HCl/MeOH) to yield the end-product as colorless oil (50 mg, 49% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.83 (m, 3H), 7.72 (d, *J* = 7.9 Hz, 1H), 7.57 – 7.51(m, 1H), 7.51 – 7.43 (m, 2H), 7.18 (t, *J* = 7.8 Hz, 1H), 6.89 (d, *J* = 7.5 Hz, 1H), 3.97 – 3.88 (m, 1H), 3.79 (d, *J* = 15.0 Hz, 1H), 3.61 (d, *J* = 15.0 Hz, 1H), 2.96 – 2.88 (m, 1H), 2.83 – 2.71 (m, 4H), 2.66 (dd, *J* = 12.0, 3.4 Hz, 1H), 2.62-2.51 (m, 2H), 2.47 (dd, *J* = 12.5, 3.4 Hz, 1H), 2.42 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.4, 135.1, 134.9, 134.4, 131.5, 128.4, 126.7, 126.1, 126.0, 123.5, 121.2, 65.5, 61.2, 55.7, 55.2, 50.2, 36.0, 24.5. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{26}N_3O_2$ 340.2020, found 340.2026. Retention time 1.96 min, HPLC purity = 100%.

1-(2-(2-hydroxy-3-(methylamino)propyl)-1,2,3,4-tetrahydroisoquinolin-5-yl)-3-phenylurea (**25**). A suspension of *tert*-butyl (3-(5-bromo-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-((*tert*-butyldimethylsilyl)oxy)propyl)(methyl)carbamate **52** (200 mg, 0.39 mmol) in 6 mL THF was added phenylurea (80 mg, 0.59 mmol), $Pd(OAc)_2$ (4 mg, 0.02

mmol), *t*-BuBrettPhos (19 mg, 0.04 mmol), and Cs_2CO_3 (192 mg, 0.59mmol). The system was purged with argon three times, then heated at 70 °C for 12 h, cooled down to room temperature, filtered through silica gel and washed with EA. The combined filtrated was concentrated and resulting residue was purified by column chromatography on silica gel to afford the intermediate. Boc protecting group was removed using general procedure (HCl/MeOH) to yield the end-product as white solid (9 mg, 7% over two steps). ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.49 (d, J = 7.7 Hz, 1H), 7.42 (dd, J = 8.6, 1.0 Hz, 2H), 7.30 – 7.22 (m, 2H), 7.14 (t, J = 7.8 Hz, 1H), 7.00 (t, J = 7.4 Hz, 1H), 6.87 (d, J = 8.1 Hz, 1H), 4.18-4.10 (m, 1H), 3.85 – 3.73 (m, 2H), 3.34 (s, 2H), 3.21 (dd, J = 12.6, 3.4 Hz, 1H), 3.01 (dd, J = 12.6, 8.7 Hz, 1H), 2.99 – 2.90(m, 2H), 2.84 (t, J = 5.8 Hz, 2H), 2.71 (s, 3H), 2.71 – 2.64(m, 2H). ^{13}C NMR (126 MHz, MeOH-d_4) δ 153.4, 138.2, 135.1, 133.6, 127.4, 125.6, 124.8, 121.5, 121.4, 120.4, 117.8, 62.9, 60.2, 55.2, 52.0, 50.2, 31.4, 23.2. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{27}N_4O_2$ 355.2129, found 355.2130. Retention time 2.10 min, HPLC purity > 95%.

N-(2-(2-hydroxy-3-(methylamino)propyl)-1,2,3,4-tetrahydroisoquinolin-5-yl)benzenesulfonamide (**26**). A suspension of *tert*-butyl (3-(5-bromo-3,4-dihydro-isoquinolin-2(1*H*)-yl)-2-((*tert*-butyldimethylsilyl)oxy)propyl)(methyl)-carbamate **52** (160 mg, 0.31 mmol) in 5 mL of acetonitrile was added benzenesulfonamide (59 mg, 0.37 mmol), CuI (4 mg, 0.02 mmol), N,N'-dimethyl-1,2-ethandiamine (17 mg, 0.20 mmol), and K_2CO_3 (127 mg, 0.94 mmol). The system was purged with argon three times, then heated at 80 °C for 12 h, cooled down to room temperature, filtered through silica gel and washed with EA. The combined filtrated was concentrated and resulting

residue was purified by column chromatography on silica gel to afford the intermediate. Boc protecting group was removed using general procedure (HCl/MeOH) to yield the end-product as colorless oil (8 mg, 8% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.2 Hz, 2H), 7.58 (t, *J* = 7.5 Hz, 1H), 7.46 (t, *J* = 7.5 Hz, 2H), 7.15 – 7.05 (m, 2H), 6.87 (d, *J* = 6.7 Hz, 1H), 3.98 (s, 1H), 3.71 (d, *J* = 14.9 Hz, 1H), 3.56 (d, *J* = 14.9 Hz, 1H), 3.16 (brs, 2H), 2.80 – 2.70 (m, 2H), 2.68 – 2.54 (m, 3H), 2.54-2.48 (m, 2H), 2.51 (s, 3H), 2.47 – 2.41 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 139.8, 135.9, 134.2, 133.0, 129.0, 128.4, 127.1, 126.4, 124.8, 122.4, 65.7, 61.3, 56.1, 55.3, 50.5, 36.1, 24.5. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{26}N_3O_3S$ 376.1689, found 376.1700. Retention time 2.12 min, HPLC purity = 100%.

1-(5-(2-fluorophenoxy)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methylamino)-propan-2-ol (**27**). The title compound was obtained as a colorless oil using a method similar to that described for compound **20** in 33% yield over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 – 7.11 (m, 1H), 7.09 – 6.99 (m, 3H), 6.95 – 6.87 (m, 1H), 6.79 (d, *J* = 7.4 Hz, 1H), 6.61 (d, *J* = 8.0 Hz, 1H), 4.01 – 3.92 (m, 1H), 3.81 (d, *J* = 15.1 Hz, 1H), 3.64 (d, *J* = 15.1 Hz, 1H), 2.98 – 2.81 (m, 4H), 2.78 – 2.72 (m, 1H), 2.69 (dd, *J* = 12.0, 3.4 Hz, 1H), 2.65 – 2.54 (m, 2H), 2.50 (dd, *J* = 12.4, 3.6 Hz, 1H), 2.45 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.6, 153.9(d, *J* = 248.9 Hz), 144.2 (d, *J* = 11.3 Hz), 136.7, 126.5, 125.2, 124.6 (d, *J* = 3.9 Hz), 124.2 (d, *J* = 6.9 Hz), 121.7, 120.8, 117.0 (d, *J* = 18.1 Hz), 114.5, 65.9, 61.7, 56.0, 55.6, 50.8, 36.3, 23.7. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{24}FN_2O_2$ 331.1816, found 331.1818. Retention time 2.33 min, HPLC purity > 98%.

1-(5-(3-fluorophenoxy)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methylamino)-propan-2-ol (**28**). The title compound was obtained as a colorless oil using a method similar to that described for compound **20** in 40% yield over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (td, *J* = 8.3, 6.7 Hz, 1H), 7.12 (t, *J* = 7.8 Hz, 1H), 6.87 (d, *J* = 7.3 Hz, 1H), 6.79 (d, *J* = 8.0 Hz, 1H), 6.73 (tdd, *J* = 8.3, 2.4, 0.8 Hz, 1H), 6.68 (dd, *J* = 8.3, 2.3 Hz, 1H), 6.60 (dt, *J* = 10.4, 2.4 Hz, 1H), 3.99 – 3.90 (m, 1H), 3.82 (d, *J* = 15.1 Hz, 1H), 3.65 (d, *J* = 15.1 Hz, 1H), 2.91 – 2.84 (m, 1H), 2.80 – 2.74 (m, 2H), 2.73 – 2.65 (m, 3H), 2.63 – 2.54 (m, 2H), 2.49 (dd, *J* = 12.4, 3.5 Hz, 1H), 2.45 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.7 (d, *J* = 246.3 Hz), 159.1 (d, *J* = 10.6 Hz), 153.4, 137.1, 130.1 (d, *J* = 9.8 Hz), 126.9, 126.8, 122.9, 117.7, 112.9, 109.4 (d, *J* = 21.3 Hz), 104.9 (d, *J* = 24.8 Hz), 66.0, 61.9, 56.1, 55.7, 50.8, 36.2, 23.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{24}FN_2O_2$ 331.1816, found 331.1809. Retention time 2.42 min, HPLC purity > 99%.

1-(5-(4-fluorophenoxy)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methylamino)-propan-2-ol (**29**). The title compound was obtained as a colorless oil using a method similar to that described for compound **20** in 42% yield over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (t, *J* = 7.9 Hz, 1H), 7.02 – 6.95 (m, 2H), 6.91 – 6.85 (m, 2H), 6.80 (d, *J* = 7.5 Hz, 1H), 6.65 (d, *J* = 7.8 Hz, 1H), 4.02 – 3.90 (m, 1H), 3.81 (d, *J* = 15.0 Hz, 1H), 3.64 (d, *J* = 15.0 Hz, 1H), 2.94 – 2.85 (m, 1H), 2.86 – 2.78 (m, 2H), 2.75 – 2.65 (m, 3H), 2.64 – 2.54 (m, 3H), 2.49 (dd, *J* = 12.4, 3.5 Hz, 1H), 2.45 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.5 (d, *J* = 241.0 Hz), 154.8, 153.2 (d, *J* = 2.4 Hz), 136.8, 126.6, 125.9, 121.8, 119.4 (d, *J* = 8.2 Hz), 116.2 (d, *J* = 23.3 Hz), 115.9, 66.0, 61.8, 56.0, 55.7,

50.8, 36.4, 23.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{24}FN_2O_2$ 331.1816, found 331.1814. Retention time 2.39 min, HPLC purity > 98%.

1-(5-(4-chlorophenoxy)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methylamino)-propan-2-ol (**30**). The title compound was obtained as a colorless oil using a method similar to that described for compound **20** in 52% yield over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 – 7.21 (m, 2H), 7.10 (t, *J* = 7.8 Hz, 1H), 6.87 – 6.81 (m, 3H), 6.72 (d, *J* = 8.0 Hz, 1H), 3.99 – 3.90 (m, 1H), 3.81 (d, *J* = 15.1 Hz, 1H), 3.64 (d, *J* = 15.1 Hz, 1H), 2.92 – 2.83 (m, 1H), 2.80 – 2.74 (m, 2H), 2.73 – 2.64 (m, 3H), 2.64 – 2.53 (m, 3H), 2.49 (dd, *J* = 12.4, 3.5 Hz, 1H), 2.44 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.2, 153.9, 136.9, 129.7, 127.6, 126.8, 126.4, 122.4, 118.8, 116.9, 65.9, 61.8, 56.0, 55.7, 50.7, 36.3, 23.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{24}ClN_2O_2$ 347.1521, found 347.1512. Retention time 2.53 min, HPLC purity > 97%.

4-((2-(2-hydroxy-3-(methylamino)propyl)-1,2,3,4-tetrahydroisoquinolin-5-yl)oxy)benzonitrile (**31**). The title compound was obtained as a colorless oil using a method similar to that described for compound **20** in 9% yield over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 – 7.52 (m, 2H), 7.17 (t, *J* = 7.8 Hz, 1H), 6.98 – 6.89 (m, 3H), 6.83 (d, *J* = 7.9 Hz, 1H), 4.01 – 3.91 (m, 1H), 3.83 (d, *J* = 15.1 Hz, 1H), 3.66 (d, *J* = 15.1 Hz, 1H), 2.91 – 2.77 (m, 1H), 2.75 – 2.66 (m, 4H), 2.64 – 2.54 (m, 4H), 2.50 (dd, *J* = 12.5, 3.5 Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.5, 152.1, 137.4, 134.2, 127.2, 127.1, 123.8, 118.9, 118.5, 117.0, 105.5, 65.9, 61.7, 56.0, 55.5, 50.5, 36.3, 29.7, 23.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{24}N_3O_2$ 338.1863, found 338.1854. Retention time 2.28 min, HPLC purity > 95%.

1-(5-(3-aminophenoxy)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methylamino)-propan-2-ol (**32**). The title compound was obtained as a colorless oil using a method similar to that described for compound **20** in 45% yield over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (t, *J* = 7.5 Hz, 1H), 7.05 (t, *J* = 8.0 Hz, 1H), 6.82 (d, *J* = 7.5 Hz, 1H), 6.78 (d, *J* = 8.0 Hz, 1H), 6.37 (ddd, *J* = 8.0, 2.3, 0.8 Hz, 1H), 6.31 (ddd, *J* = 8.0, 2.3, 0.8 Hz, 1H), 6.23 (t, *J* = 2.2 Hz, 1H), 3.99 – 3.92 (m, 1H), 3.82 (d, *J* = 15.0 Hz, 1H), 3.66 (brs, 2H), 3.64 (d, *J* = 15.0 Hz, 2H), 2.92 – 2.85 (m, 1H), 2.84 – 2.75 (m, 2H), 2.75 – 2.64 (m, 2H), 2.63 – 2.54 (m, 3H), 2.49 (dd, *J* = 12.4, 3.5 Hz, 2H), 2.45 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.7, 154.1, 148.0, 136.6, 130.3, 126.6, 126.5, 122.0, 117.3, 109.6, 107.7, 104.3, 66.0, 61.8, 56.1, 55.7, 50.8, 36.4, 23.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{26}N_3O_2$ 328.202, found 328.2029. Retention time 1.68 min, HPLC purity > 99%.

1-(5-(3-chloro-4-fluorophenoxy)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methyl-amino)propan-2-ol (**33**). The title compound was obtained as a colorless oil using a method similar to that described for compound **20** in 26% yield over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (t, *J* = 7.8 Hz, 1H), 7.08 – 7.01 (m, 1H), 6.95 (dd, *J* = 5.7, 2.6 Hz, 1H), 6.85 (d, *J* = 7.6 Hz, 1H), 6.80-6.75 (m, 1H), 6.70 (d, *J* = 8.0 Hz, 1H), 4.00 – 3.90 (m, 1H), 3.81 (d, *J* = 15.2 Hz, 1H), 3.64 (d, *J* = 15.1 Hz, 1H), 2.92 – 2.84 (dt, *J* = 10.6, 5.4 Hz, 1H), 2.80 – 2.74 (m, 2H), 2.73 – 2.65 (m, 3H), 2.63 – 2.53 (m, 3H), 2.49 (dd, *J* = 12.5, 3.0 Hz, 1H), 2.44 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.93 (d, *J* = 242.8 Hz), 153.87, 153.6 (d, *J* = 2.7 Hz), 137.1, 126.8, 126.3, 122.6, 121.5 (d, *J* = 19.4 Hz), 119.5, 117.1, 117.0 (d, *J* = 17.0 Hz), 116.7, 65.9, 61.8, 56.0, 55.6, 50.7,

36.2, 23.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{23}ClFN_2O_2$ 365.1386, found 365.1384. Retention time 2.58 min, HPLC purity > 96%.

1-(5-(3,5-dimethylphenoxy)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methyl-amino)propan-2-ol (**34**). The title compound was obtained as a colorless oil using a method similar to that described for compound **20** in 32% yield over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (t, *J* = 7.8 Hz, 1H), 6.82 (d, *J* = 7.8 Hz, 1H), 6.76 – 6.68 (m, 2H), 6.55 (s, 2H), 4.04 – 3.94 (m, 1H), 3.83 (d, *J* = 14.7 Hz, 1H), 3.66 (d, *J* = 14.9 Hz, 1H), 2.93 – 2.85 (m, 1H), 2.84 – 2.77 (m, 2H), 2.75 – 2.67 (m, 2H), 2.64 – 2.48 (m, 3H), 2.47 (s, 3H), 2.27 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.5, 154.5, 139.6, 136.7, 126.6, 126.3, 124.6, 121.8, 116.8, 115.6, 66.0, 61.9, 56.2, 55.7, 50.9, 36.3, 23.9, 21.4. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{29}N_2O_2$ 341.2224, found 341.2228. Retention time 2.62 min, HPLC purity > 99%.

1-(methylamino)-3-(5-(4-(thiazol-2-yl)phenoxy)-3,4-dihydroisoquinolin-2(1H)-yl)propan-2-ol (**35**). The title compound was obtained as a colorless oil using a method similar to that described for compound **20** in 24% yield over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 – 7.87 (m, 2H), 7.82 (d, *J* = 3.3 Hz, 1H), 7.28 (d, *J* = 3.3 Hz, 1H), 7.15 (t, *J* = 7.8 Hz, 1H), 7.00 – 6.93 (m, 2H), 6.89 (d, *J* = 7.6 Hz, 1H), 6.83 (d, *J* = 7.6 Hz, 1H), 4.00 – 3.92 (m, 1H), 3.84 (d, *J* = 15.1 Hz, 1H), 3.66 (d, *J* = 15.1 Hz, 1H), 2.93-2.86 (m, 1H), 2.83 – 2.76 (m, 2H), 2.75 – 2.66 (m, 2H), 2.65 – 2.54 (m, 2H), 2.51 (dd, *J* = 12.4, 3.5 Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.9, 159.3, 153.4, 143.5, 137.0, 128.24, 128.18, 126.83, 126.75, 122.8, 118.3, 117.7, 117.0, 65.9, 61.8,

56.0, 55.6, 50.7, 36.2, 23.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{22}H_{26}N_3O_2S$ 396.1740, found 396.1738. Retention time 2.40 min, HPLC purity > 99%.

1-(methylamino)-3-(5-(3-(thiazol-2-yl)phenoxy)-3,4-dihydroisoquinolin-2(1H)-yl)propan-2-ol (**36**). The title compound was obtained as a colorless oil using a method similar to that described for compound **22** in 25% yield over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 3.2 Hz, 1H), 7.63 (d, *J* = 7.8 Hz, 1H), 7.58 (d, *J* = 1.5 Hz, 1H), 7.35 (t, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 3.2 Hz, 1H), 7.11 (t, *J* = 7.8 Hz, 1H), 6.95 (dd, *J* = 8.2, 2.3 Hz, 1H), 6.84 (d, *J* = 7.6 Hz, 1H), 6.78 (d, *J* = 8.0 Hz, 1H), 4.00 – 3.90 (m, 1H), 3.82 (d, *J* = 15.1 Hz, 1H), 3.65 (d, *J* = 15.0 Hz, 1H), 2.94-2.86 (m,1H), 2.84 – 2.78 (m, 2H), 2.75 – 2.54 (m,5H), 2.56 – 2.46 (m, 1H), 2.45 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.7, 158.1, 153.9, 143.8, 136.9, 135.3, 130.3, 126.8, 126.4, 122.4, 121.1, 119.2, 119.1, 117.0, 115.7, 65.7, 61.7, 56.1, 55.5, 50.8, 36.0, 23.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{22}H_{26}N_3O_2S$ 396.1740, found 396.1743. Retention time 2.37 min, HPLC purity > 95%.

1-(methylamino)-3-(6-phenoxy-3,4-dihydroisoquinolin-2(1H)-yl)propan-2-ol (**37**). The title compound was obtained as a colorless oil from *tert*-butyl 6-bromo-3,4-dihydroisoquinoline-2(1H)-carboxylate (**53b**) using a method similar to that described for compound **20** in 40% yield over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.30 (m, 2H), 7.13 – 7.06 (m, 1H), 7.05 – 6.97 (m, 3H), 6.82 (dd, *J* = 8.3, 2.5 Hz, 1H), 6.78 (d, *J* = 2.4 Hz, 1H), 4.02 – 3.94 (m, 1H), 3.80 (d, *J* = 14.6 Hz, 1H), 3.62 (d, *J* = 14.6 Hz, 1H), 2.97 – 2.84 (m, 3H), 2.76 – 2.67 (m, 3H), 2.67 – 2.58 (m, 3H), 2.53 (dd, *J* = 12.4, 3.6 Hz, 1H), 2.48 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.5, 155.4, 135.8,

129.7, 129.5, 127.8, 123.0, 118.8, 118.6, 116.9, 66.0, 61.9, 55.80, 55.78, 51.0, 36.5, 29.2. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{25}N_2O_2$ 313.1911, found 313.1900. Retention time 2.34 min, HPLC purity > 96%.

1-(5-((2-fluorobenzyl)oxy)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methylamino)-propan-2-ol (**38**). The title compound was obtained as a colorless oil using a method similar to that described for compound **21** in 43% over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (t, *J* = 7.5 Hz, 1H), 7.34 – 7.27 (m, 1H), 7.16 (t, *J* = 7.5 Hz, 1H), 7.13 – 7.03 (m, 2H), 6.77 (d, *J* = 8.1 Hz, 1H), 6.67 (d, *J* = 7.7 Hz, 1H), 5.13 (s, 2H), 3.96 (ddt, *J* = 10.3, 7.0, 3.4 Hz, 1H), 3.79 (d, *J* = 14.9 Hz, 1H), 3.61 (d, *J* = 14.9 Hz, 1H), 2.97 – 2.90 (m, 1H), 2.89 – 2.83 (m, 2H), 2.76 – 2.71 (m, 1H), 2.69 (dd, *J* = 11.9, 3.3 Hz, 2H), 2.64 – 2.55 (m, 2H), 2.50 (dd, *J* = 12.4, 3.4 Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.4 (d, *J* = 247.5 Hz), 156.2, 136.1, 129.6(d, *J* = 8.1 Hz), 129.4, 126.5, 124.6 (d, *J* = 14.2 Hz), 124.3, 123.7, 119.3, 115.4 (d, *J* = 21.2 Hz), 108.9, 65.9, 63.7, 63.6, 61.7, 56.2, 55.7, 51.0, 36.4, 23.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{26}FN_2O_2$ 345.1973, found 345.1978. Retention time 2.29 min, HPLC purity > 95%.

1-(5-((3-fluorobenzyl)oxy)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methylamino)-propan-2-ol (**39**). The title compound was obtained as a colorless oil using a method similar to that described for compound **21** in 36% over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.29 (m, 1H), 7.20-7.12 (m, 2H), 7.09 (t, *J* = 7.9 Hz, 1H), 7.00 (td, *J* = 8.5, 2.2 Hz, 1H), 6.73 – 6.62 (m, 2H), 5.05 (s, 2H), 3.96 (ddd, *J* = 10.3, 7.1, 3.5 Hz, 1H), 3.78 (d, *J* = 14.9 Hz, 1H), 3.60 (d, *J* = 14.9 Hz, 1H), 2.97 – 2.90 (m, 1H), 2.88 – 2.85 (m, 2H), 2.77 – 2.72 (m, 1H), 2.69 (dd, *J* = 11.9, 3.3 Hz, 1H), 2.59 (dt, *J* = 11.9,

8.5 Hz, 2H), 2.49 (dd, *J* = 12.4, 3.5 Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.0 (d, *J* = 247.5 Hz), 156.0, 140.0 (d, *J* = 7.3 Hz), 136.2, 130.1 (d, *J* = 7.3 Hz), 126.3, 123.6, 122.4, 119.2, 114.6 (d, *J* =21.0 Hz), 113.9 (d, *J* =21.0 Hz), 108.8, 68.9, 65.9, 61.7, 56.1, 55.7, 50.9, 36.4, 23.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{26}FN_2O_2$ 345.1973, found 345.1975. Retention time 2.31 min, HPLC purity > 96%.

1-(5-((4-fluorobenzyl)oxy)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methylamino)-propan-2-ol (**40**). The title compound was obtained as a colorless oil using a method similar to that described for compound **21** in 49% over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.36 (m, 2H), 7.12 – 7.03 (m, 3H), 6.72 (d, *J* = 8.1 Hz, 1H), 6.67 (d, *J* = 7.7 Hz, 1H), 5.02 (s, 2H), 3.96 (ddt, *J* = 10.3, 7.1, 3.4 Hz, 1H), 3.78 (d, *J* = 14.9 Hz, 1H), 3.60 (d, *J* = 15.0 Hz, 1H), 2.95 – 2.88 (m, 1H), 2.84 (d, *J* = 2.0 Hz, 2H), 2.76 – 2.71 (m, 1H), 2.69 (dd, *J* = 11.9, 3.1 Hz, 1H), 2.64 – 2.55 (dt, *J* = 12.0, 7.6 Hz, 2H), 2.49 (dd, *J* = 12.5, 3.5 Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.5 (d, *J* = 246.5 Hz), 156.3, 136.2, 133.18, 133.16, 129.0 (d, J = 8.2 Hz), 126.4, 123.7, 119.2, 115.54 (d, *J* = 21.5 Hz), 108.9, 69.2, 66.0, 61.8, 56.2, 55.8, 51.0, 36.6, 23.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{26}FN_2O_2$ 345.1973, found 345.1973. Retention time 2.31 min, HPLC purity > 97%.

1-(5-((4-chlorobenzyl)oxy)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methylamino)-propan-2-ol (**41**). The title compound was obtained as a white solid using a method similar to that described for compound **21** in 23% over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.32 (m, 4H), 7.09 (t, *J* = 7.9 Hz, 1H), 6.70 (d, *J* = 7.9 Hz, 2H), 6.67 (d, *J* = 7.9 Hz, 2H), 5.02 (s, 2H), 4.01 – 3.92 (m, 1H), 3.78 (d, *J* = 15.0 Hz, 1H), 3.60 (d, *J*

= 15.0 Hz, 1H), 2.96-2.90 (m, 1H), 2.84 (d, *J* = 2.2 Hz, 2H), 2.77 – 2.72 (m, 1H), 2.69 (dd, *J* = 12.1, 3.6 Hz, 2H), 2.65 – 2.55 (m, 3H), 2.49 (dd, *J* = 12.4, 3.4 Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.1, 136.2, 135.9, 133.6, 128.8, 128.5, 126.4, 123.6, 119.3, 108.9, 69.0, 66.0, 61.8, 56.2, 55.8, 51.0, 36.5, 23.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{26}ClN_2O_2$ 361.1677, found 361.1681. Retention time 2.45 min, HPLC purity > 99%.

4-(((2-(2-hydroxy-3-(methylamino)propyl)-1,2,3,4-tetrahydroisoquinolin-5-yl)oxy)-methyl)benzonitrile (**42**). The title compound was obtained as a white solid using a method similar to that described for compound **21** in 15% over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 8.2 Hz, 2H), 7.53 (d, *J* = 8.2 Hz, 2H), 7.08 (t, *J* = 7.9 Hz, 1H), 6.71 – 6.64 (t, *J* = 7.8 Hz, 2H), 5.12 (s, 2H), 4.00-3.92 (m, 1H), 3.79 (d, *J* = 15.0 Hz, 1H), 3.61 (d, *J* = 15.0 Hz, 1H), 2.97 – 2.91 (m, 1H), 2.89 – 2.83 (m, 2H), 2.78 – 2.73 (m, 1H), 2.69 (dd, *J* = 12.0, 3.4 Hz, 1H), 2.65 – 2.55 (m, 3H), 2.50 (dd, *J* = 12.4, 3.4 Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.8, 142.9, 136.4, 132.5, 127.4, 126.5, 123.6, 119.6, 118.8, 111.7, 108.8, 68.7, 66.0, 61.7, 56.2, 55.8, 50.9, 36.5, 23.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{26}N_3O_2$ 352.2020, found 352.2025. Retention time 2.20 min, HPLC purity > 96%.

1-(5-((4-(tert-butyl)benzyl)oxy)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methyl-amino)propan-2-ol (**43**). The title compound was obtained as a colorless oil using a method similar to that described for compound **21** in 27% over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43-7.35 (m, 4H), 7.10 (t, *J* = 7.9 Hz, 1H), 6.76 (d, *J* = 8.1 Hz, 1H), 6.66 (d, *J* = 7.7 Hz, 1H), 5.04 (s, 2H), 3.96 (ddd, *J* = 10.2, 7.0, 3.4 Hz, 1H), 3.80

(d, J = 14.9 Hz, 1H), 3.61 (d, J = 14.9 Hz, 1H), 2.97 – 2.90 (m, 1H), 2.89 – 2.83 (m, 2H), 2.76-2.71 (m, 1H), 2.71 – 2.67 (m, 1H), 2.65-2.56 (m, 2H), 2.50 (dd, J = 12.4, 3.4 Hz, 1H), 2.47 (s, 3H), 1.34 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.5, 150.8, 136.0, 134.4, 127.0, 126.4, 125.5, 123.6, 118.9, 108.9, 69.6, 65.9, 61.8, 56.2, 55.7, 51.1, 36.3, 34.6, 31.4, 23.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{24}H_{35}N_2O_2$ 383.2693, found 383.2700. Retention time 2.75 min, HPLC purity > 99%.

1-(5-([1,1'-biphenyl]-4-ylmethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methyl-amino)propan-2-ol (**44**). The title compound was obtained as a white solid using a method similar to that described for compound **21** in 52% over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 – 7.59 (m, 4H), 7.51 (d, J = 8.1 Hz, 2H), 7.46 (t, J = 7.6 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 7.12 (t, J = 7.9 Hz, 1H), 6.78 (d, J = 8.1 Hz, 1H), 6.69 (d, J = 7.7 Hz, 1H), 5.12 (s, 2H), 4.03 – 3.96 (m, 1H), 3.81 (d, J = 15.0 Hz, 1H), 3.63 (d, J = 14.9 Hz, 1H), 2.99 – 2.94 (m, 1H), 2.93 – 2.90 (m, 2H), 2.79 – 2.74 (m, 1H), 2.71 (dd, J = 12.1, 3.4 Hz, 1H), 2.66-2.56 (m, 2H), 2.51 (dd, J = 12.4, 3.5 Hz, 1H), 2.48 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.4, 140.89, 140.85, 136.4, 136.1, 128.9, 127.7, 127.5, 127.4, 127.2, 126.4, 123.7, 119.1, 109.0, 69.6, 66.1, 61.8, 56.2, 55.9, 51.0, 36.7, 24.0. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{26}H_{31}N_2O_2$ 403.2380, found 403.2387. Retention time 2.80 min, HPLC purity = 100%.

2-(4-(((2-(oxiran-2-ylmethyl)-1,2,3,4-tetrahydroisoquinolin-5-yl)oxy)methyl)-phenyl)thiazole (**45a**). A suspension *tert*-butyl 5-((4-(thiazol-2-yl)benzyl)oxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate **56a** (180 mg, 0.44 mmol) in MeOH (5 mL) was added HCl/dioxane (4 M solution, 2 mL). The solution was stirred at room temperature

for 2 h prior to removal of all solvents under reduced pressure. The residue was added saturated $NaHCO_3$ aqueous solution and extracted with DCM. The organic was dried with anhydrous Na_2SO_4 and removed all solvents under reduced pressure to afford the intermediate as white solid without further purification. The intermediate was dissolved in THF (6 mL) and then added $KF{\cdot}2H_2O$ (83 mg, 0.88 mmol) and 2-(bromomethyl)oxirane (120 mg, 0.88 mmol). The result solution was stirred at room temperature overnight, filtered and concentrated and resulting residue was purified by column chromatography on silica gel to afford the product as white solid (150 mg, 90% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.2 Hz, 2H), 7.86 (d, *J* = 3.3 Hz, 1H), 7.49 (d, *J* = 8.2 Hz, 2H), 7.32 (d, *J* = 3.3 Hz, 1H), 7.08 (t, *J* = 7.9 Hz, 1H), 6.70 (t, *J* = 7.9 Hz, 2H), 5.11 (s, 2H), 3.80 (d, *J* = 14.9 Hz, 1H), 3.67 (d, *J* = 15.0 Hz, 1H), 3.20 (td, *J* = 6.7, 3.4 Hz, 1H), 2.96 – 2.85 (m, 5H), 2.84-2.79 (m, 2H), 2.55 (dd, *J* = 5.0, 2.7 Hz, 1H), 2.45 (dd, *J* = 13.3, 6.7 Hz, 1H). MS(ESI) m/z 379.3 $[M+H]^+$.

1-(methylamino)-3-(5-((4-(thiazol-2-yl)benzyl)oxy)-3,4-dihydroisoquinolin-2(1H)-yl)propan-2-ol (**45**). A suspension 2-(4-(((2-(oxiran-2-ylmethyl)-1,2,3,4-tetrahydro-isoquinolin-5-yl)oxy)methyl)phenyl)thiazole **45a** (150 mg, 0.40 mmol) in MeOH (4 mL) was slowly added a 30% solution of $MeNH_2$ in MeOH (0.5 mL). The result solution was stirred at room temperature for 12 h, concentrated and resulting residue was purified by column chromatography on silica gel to afford the product as white solid (144 mg, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.3 Hz, 2H), 7.86 (d, *J* = 3.3 Hz, 1H), 7.50 (d, *J* = 8.2 Hz, 2H), 7.32 (d, *J* = 3.3 Hz, 1H), 7.09 (t, *J* = 7.9 Hz, 1H), 6.72 (d, *J* = 8.1 Hz, 1H), 6.67 (d, *J* = 7.7 Hz, 1H), 5.11 (s, 2H), 4.02 – 3.91

(m, 1H), 3.79 (d, *J* = 14.9 Hz, 1H), 3.61 (d, *J* = 14.9 Hz, 1H), 2.97 – 2.92 (m, 1H), 2.91 – 2.85 (m, 2H), 2.75 (dd, *J* = 11.1, 5.4 Hz, 1H), 2.69 (dd, *J* = 12.0, 3.5 Hz, 1H), 2.64 – 2.56 (ddd, *J* = 16.6, 10.9, 5.8 Hz, 2H), 2.50 (dd, *J* = 12.4, 3.5 Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.1, 156.1, 143.8, 139.4, 136.1, 133.1, 127.5, 126.8, 126.3, 123.6, 119.2, 118.9, 108.9, 69.3, 66.1, 61.7, 56.1, 55.8, 50.9, 36.6, 23.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{23}H_{28}N_3O_2S$ 410.1897, found 410.1888. Retention time 2.36 min, HPLC purity = 100%.

1-(methylamino)-3-(5-((3-(thiazol-2-yl)benzyl)oxy)-3,4-dihydroisoquinolin-2(1H)-yl)propan-2-ol (**46**). The title compound was obtained as a colorless oil using a method similar to that described for compound **45** in 34% yield over five steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.90 (d, *J* = 7.5 Hz, 1H), 7.87 (d, *J* = 3.2 Hz, 1H), 7.50 (d, *J* = 7.7 Hz, 1H), 7.45 (t, *J* = 7.6 Hz, 1H), 7.33 (d, *J* = 3.3 Hz, 1H), 7.09 (t, *J* = 7.9 Hz, 1H), 6.74 (d, *J* = 8.1 Hz, 1H), 6.66 (d, *J* = 7.7 Hz, 1H), 5.12 (s, 2H), 4.02 – 3.90 (m, 1H), 3.79 (d, *J* = 15.0 Hz, 1H), 3.61 (d, *J* = 14.9 Hz, 1H), 2.97 – 2.91 (m, 1H), 2.91 – 2.85 (m, 2H), 2.74 (dd, *J* = 11.0, 5.2 Hz, 1H), 2.68 (dd, *J* = 12.0, 3.5 Hz, 1H), 2.64 – 2.55 (m, 2H), 2.49 (dd, *J* = 12.4, 3.5 Hz, 1H), 2.45 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.1, 156.1, 143.8, 138.4, 136.0, 133.8, 129.3, 128.6, 126.4, 126.1, 125.2, 123.6, 119.2, 119.0, 108.9, 69.3, 65.7, 61.7, 56.1, 55.5, 51.0, 36.1, 23.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{23}H_{28}N_3O_2S$ 410.1897, found 410.1889. Retention time 1.86 min, HPLC purity = 100%.

1-(6-(benzyloxy)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(methylamino)propan-2-ol (**47**). The title compound was obtained as a colorless oil from *tert*-butyl 6-hydroxy-

3,4-dihydroisoquinoline-2(1*H*)-carboxylate (**54b**) using a method similar to that described for compound **21** in 21% yield over four steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.35 (m, 4H), 7.34-7.29 (m, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 6.78 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.73 (d, *J* = 2.5 Hz, 1H), 5.03 (s, 3H), 4.01 – 3.92 (m, 1H), 3.74 (d, *J* = 14.4 Hz, 1H), 3.56 (d, *J* = 14.4 Hz, 1H), 2.96 – 2.83 (m, 3H), 2.77 – 2.64 (m, 3H), 2.64 – 2.54 (m, 2H), 2.49 (dd, *J* = 12.4, 3.6 Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.3, 137.2, 135.4, 128.6, 128.0, 127.6, 127.5, 127.0, 114.4, 113.1, 70.1, 65.8, 61.9, 55.8, 55.6, 51.2, 36.1, 29.4. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{27}N_2O_2$ 327.2067, found 327.2064. Retention time 2.38 min, HPLC purity > 99%.

(S)-1-(methylamino)-3-(5-((4-(thiazol-2-yl)benzyl)oxy)-3,4-dihydroisoquinolin-2(1H)-yl)propan-2-ol (**48**). The title compound was obtained as a white solid from S-glycidylnosylate using a method similar to that described for compound **45** in 45% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, *J* = 8.4 Hz, 2H), 7.87 (d, *J* = 3.2 Hz, 1H), 7.51 (d, *J* = 8.5 Hz, 2H), 7.34 (d, *J* = 3.3 Hz, 1H), 7.10 (t, *J* = 8.0 Hz, 1H), 6.73 (d, J = 8.1 Hz, 1H), 6.68 (d, J = 7.7 Hz, 1H), 5.12 (s, 2H), 4.02 – 3.91 (m, 1H), 3.81 (d, *J* = 14.8 Hz, 1H), 3.62 (d, *J* = 14.7 Hz, 1H), 3.00 – 2.92 (m, 1H), 2.91 – 2.85 (m, 2H), 2.75 (dd, *J* = 11.1, 5.9 Hz, 1H), 2.70 (dd, *J* = 11.9, 3.5 Hz, 1H), 2.66 – 2.56 (m, 2H), 2.51 (dd, *J* = 12.5, 3.4 Hz, 1H), 2.47 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.1, 156.1, 143.8, 139.4, 136.1, 133.1, 127.5, 126.8, 126.3, 123.6, 119.2, 118.9, 108.9, 69.3, 66.1, 61.6, 56.1, 55.8, 50.9, 36.7, 23.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{23}H_{28}N_3O_2S$ 410.1897, found 410.1902. Retention time 2.36 min, HPLC purity > 99 %.

(R)-1-(methylamino)-3-(5-((4-(thiazol-2-yl)benzyl)oxy)-3,4-dihydroisoquinolin-

2(1H)-yl)propan-2-ol (**49**). The title compound was obtained as a white solid from R-glycidylnosylate using a method similar to that described for compound **45** in 48% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, *J* = 8.4 Hz, 2H), 7.87 (d, *J* = 3.2 Hz, 1H), 7.51 (d, *J* = 8.5 Hz, 2H), 7.34 (d, *J* = 3.3 Hz, 1H), 7.10 (t, *J* = 8.0 Hz, 1H), 6.73 (d, J = 8.1 Hz, 1H), 6.68 (d, J = 7.7 Hz, 1H), 5.12 (s, 2H), 4.02 – 3.91 (m, 1H), 3.81 (d, *J* = 14.8 Hz, 1H), 3.62 (d, *J* = 14.7 Hz, 1H), 3.00 – 2.92 (m, 1H), 2.91 – 2.85 (m, 2H), 2.75 (dd, *J* = 11.1, 5.9 Hz, 1H), 2.70 (dd, *J* = 11.9, 3.5 Hz, 1H), 2.66 – 2.56 (m, 2H), 2.51 (dd, *J* = 12.5, 3.4 Hz, 1H), 2.47 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.1, 156.1, 143.8, 139.4, 136.1, 133.1, 127.5, 126.8, 126.3, 123.6, 119.2, 118.9, 108.9, 69.3, 66.1, 61.6, 56.1, 55.8, 50.9, 36.7, 23.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{23}H_{28}N_3O_2S$ 410.1897, found 410.1904. Retention time 2.37 min, HPLC purity = 100%.

Tert-butyl(3-(5-bromo-3,4-dihydroisoquinolin-2(1H)-yl)-2-hydroxypropyl)-(methyl)carbamate (**51**). A suspension of 5-bromo-1,2,3,4-tetrahydroisoquinoline **12a** (1.5 g, 7.0 mmol) in *i*-PrOH (40 mL) was added *tert*-butyl methyl(oxiran-2-ylmethyl)carbamate **50** (1.9 g, 10.5 mmol). The reaction solution was heated at 80 °C overnight before being concentrated and purified by column chromatography on silica gel to provide the desired product as colorless oil (2.1 g, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, *J* = 7.3 Hz, 1H), 7.03 – 6.92 (m, 2H), 4.03 – 3.92 (m, 1H), 3.76 (d, *J* = 15.0 Hz, 1H), 3.58 (d, *J* = 15.0 Hz, 1H), 3.52 – 3.34 (m, 2H), 3.28 – 3.12 (m, 1H), 2.96 (s, 3H), 2.94 – 2.69(d, *J* = 8.4 Hz, 4H), 1.46 (s, 9H). MS (ESI) m/z 399.1 $[M+H]^+$.

Tert-butyl(3-(5-bromo-3,4-dihydroisoquinolin-2(1H)-yl)-2-((tert-

butyldimethyl-silyl)oxy)propyl)(methyl)carbamate (**52**). A suspension of *tert*-butyl (3-(5-bromo-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-hydroxypropyl)(methyl)carbamate **51** (2.0 g, 5.0 mmol)and imidazole (730 mg, 10.8 mmol) in DCM (20 mL)was added TBSCl (1.5 g, 10 mmol) slowly at room temperature. The reaction was stirred at 35 °C overnight followed by washing with water and brine. The organic layer was dried with anhydrous Na_2SO_4, filtered and concentrated and resulting residue was purified by column chromatography on silica gel to afford the product as colorless oil (900 mg, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (s, 1H), 6.98 (d, *J* = 7.7 Hz, 2H), 4.12 (dd, *J* = 14.3, 7.1 Hz, 1H), 3.67 (d, *J* = 14.3 Hz, 1H), 3.57 (s, 1H), 2.92 (d, *J* = 10.8 Hz, 3H), 2.79 (dd, *J* = 18.2, 4.6 Hz, 4H), 2.50 (d, *J* = 5.4 Hz, 2H), 1.45 (s, 8H), 0.90 (s, 8H), 0.06 (d, *J* = 8.4 Hz, 6H). MS (ESI) m/z 513.3 $[M+H]^+$.

Tert-butyl-5-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**55a**). A suspension *tert*-butyl 5-hydroxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate **54a** (980 mg, 3.94 mmol) in acetonitrile (40 mL) was added Cs_2CO_3 (1.9 g, 5.90 mmol) and 4-(bromomethyl) benzene boronic acid pinacol ester (1.4 g, 4.72 mmol). The result solution was stirred at room temperature for 4 h, filtered and concentrated and resulting residue was purified by column chromatography on silica gel to afford the product as white solid (950 mg, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.0 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.11 (s, 1H), 6.73 (d, *J* = 8.0 Hz, 2H), 5.09 (s, 2H), 4.56 (s, *J* = 3.4Hz, 2H), 3.64 (t, *J* = 3.4Hz, 2H), 2.83 (t, 2H), 1.49 (s, 9H), 1.35 (s, 12H). MS(ESI) m/z 466.3 $[M+H]^+$.

Tert-butyl-5-((4-(thiazol-2-yl)benzyl)oxy)-3,4-dihydroisoquinoline-2(1H)-

carboxylate (**56a**). A suspension of *tert*-butyl 5-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)-3,4-dihydroisoquinoline-2(1H)-carboxylate **55a** (950 mg, 2.0 mmol), 2-bromothiazole (502 mg, 3.0 mmol) and K_3PO_4 (1.27 g, 6.0 mmol) in dioxane and water (3:1, 18 mL),was treated with $Pd(Ph_3P)_4$ (138 mg, 0.12 mmol) and Ph_3P (262 mg, 1.0 mmol). The system was purged with argon three times, then heated at 90 °C for 2 h, cooled down to room temperature, filtered through silica gel and washed with EA. The combined filtrated was concentrated and resulting residue was purified by column chromatography on silica gel to afford the product as white solid (640 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, J = 8.3 Hz, 2H), 7.87 (d, J = 3.3 Hz, 1H), 7.50 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 3.3 Hz, 1H), 7.13 (t, J = 7.9 Hz, 1H), 6.75 (d, J = 8.0 Hz, 2H), 5.12 (s, 2H), 4.58 (s, 2H), 3.66 (s, 2H), 2.85 (s, 2H), 1.49 (s, 9H). MS(ESI) m/z 423.2 $[M+H]^+$.

Biological Assay Methods

Enzymatic Assays for PRMT4

The enzyme activity was determined by Alpha LISA assay in 384-well plate. The enzyme PRMT4, SAM, and histone H3(21-44) peptide was diluted in assay buffer before use. SAM was used as the methyl group donor and unmethylated histone H3 was used as a substrate. All these components were incubated with indicated compounds at room temperature for 60 min. Subsequently, anti-methyl-arginine acceptor beads were added at a final concentration of 20 μg/mL and the plate was incubated at room temperature for 60 min in subdued light. Afterward, add 10 μL Streptavidin donor beads to the enzymatic reaction at a final concentration of 20 μg/mL,

cover the plate with TopSeal-A film and incubate 30 min at room temperature. Finally, the assay was detected by using an Envision® reader. The data were analyzed with GraphPad prism.

X-ray Crystallography

The human PRMT4 (140-480) was purchased from Viva Biotech Ltd (Shanghai, China). Before crystallization, the 18.7 mg/mL PRMT4 protein was diluted to 2mg/ml with buffer (25 mM HEPES, pH 7.5, 100 mM NaCl, 1 mM TCEP), then compound **10** was added to the protein solution to give a final concentration of 2 mM. Crystallization using hanging drop vapor diffusion method at 16 °C by mixing 1 μL of the protein solution with 1 μL of the reservoir solution containing 20% PEG3350, 0.15 M sodium malate at pH 7.0. Diffraction quality crystals appeared within 1 week.

Data were collected at 100 K on beamline BL17U at the Shanghai Synchrotron Radiation Facility (SSRF) (Shanghai, China) for the cocrystallized structures. The data were processed with the HKL200032 [42] software packages, and the structures were then solved by molecular replacement, using the CCP4 program MOLREP.[43] The search model used for the crystals was the reported complex structure (PDB code 2Y1W). The structures were refined using the program REFMAC5 combined with the simulated-annealing protocol implemented in the program PHENIX.[44] With the aid of the program Coot,[45] compound, water molecules, and others were fitted into to the initial $F_0 - F_c$ maps.

Selectivity Assays

The selectivity assay was performed as previously described.[28] The selectivity against the PRMT family of enzymes including PRMT1, PRMT3, PRMT5, PRMT6, and

PRMT8 and diverse histone lysine methyltransferases including NSD2, SMYD3, and DOT1L was assessed via the AlphaLISA assay. Briefly, both enzyme and substrate solution were prepared in 1× assay buffer, while compounds were transferred to assay plate by Echo in a final concentration of 1% DMSO. 5 μL of enzyme solution was transferred to assay plate and incubated at room temperature for 15 min. Then 5 μL of substrate solution was added to each well to start the reaction. After incubation at room temperature for 60 min, 15 μL of beads mix solution was added to the assay plate and incubated at room temperature for 60 min with subdued light. The signal was collected with EnSpire and analyzed in Graphpad Prism. The selectivity against PRMT7 was assessed using the radioisotope assay as described elsewhere.[46] The selectivity against EZH2 and MLL1 was assessed using the HTRF assay. In HTFR assay, enzyme, SAM, compound and peptide substrate in assay buffer were diluted in 1× assay buffer just before use. 4 μL of compound and 2 μL of enzyme were added to the wells of a white OptiPlate-384 and incubated for 10 min at room temperature. Subsequently, 4 μL of substrate/SAM was mixed to the reaction system and incubated at room temperature for 4 hours. Then, a 2× mix of antibody at 0.225 μg/mL and SA-XL665 at 10 ng/ul were prepared, respectively. Finally, 10 μL of detection mixture (2×) was added to the plate. After incubation in subdued light for 1h at room temperature, signal was collected with an HTRF compatible reader and analyzed in GraphPad Prism.

Cell Viability Assays

Cells were seeded in 96-well plates in a volume of 200 μL and treated with indicated compound in corresponding concentration (DMSO as control). Cell viability assays

（SRB or CCK8 assay）were carried out after incubation for 6 days. The absorbance (optical density, OD) was read at a wavelength of 450 nm for CCK8 and 515 or 560 nm for SRB. The IC50 values were calculated by concentration-response curve fitting using the four-parameter method, and the data were normalized for control cells. All treatments were determined in triplicate.

Western Bolt

Total cellular extracts were prepared by adding 2% SDS with 1 mmol/L phenylmethyl sulfonyl fluoride (PMSF) to the cells on ice. In the in vivo study, the tumor tissue samples were homogenized for tissue lysate extraction. The protein concentration of cellular extracts and tissue lysate extraction were quantified both by the method of BCA protein assay, and the protein samples were separated by 8%-15% SDS-polyacrylamide gels, transferred to nitrocellulose membranes. The blots were blocked with blocking buffer (3% BSA in TBST) at room temperature for 1 h, and probed with primary antibodies at 4°C overnight. Then, the bolts were washed three times with TBST and then incubated with HRP conjugated secondary antibody at room temperature for 1h. Following three TBST washes, the blots were visualized with enhanced chemiluminescence. Primary antibodies used were as follows: anti-aDMA(Cell Signaling Technology no. 13522), anti-beta-actin(Proteintech Group no. 66009-1-lg), anti-PABP1(Cell Signaling Technology no. 4992), anti-PABP1me2a(Cell Signaling Technology no. 3505), anti-BAF155(Cell Signaling Technology no.11956), and anti-BAF155me2a(Cell Signaling Technology no. 94962)

Cell Cycle Analysis

Leukemia cell MOLM13 was seeded in six-well plates in a volume of 2 mL, and treated with concentrations of the indicated compound or DMSO. Cells were harvested 48h post-treatment, washed two times with ice cold phosphate buffered saline (PBS), and incubated in ice cold 70% ethanol/PBS (v/v) for at least 24 h. The fixed cells were then washed with room temperature PBS two times and incubated with RNase A (5 μg/ml) and propidium iodide for 30 min at room temperature. The stained cells were then analyzed by FACSCAN (BD Biosciences) and the resulting data were analyzed by FlowJo V7.6.1.

Annexin V-FITC/PI Apoptosis Assay

For cell apoptosis analysis, after treating with concentrations of the indicated compound or DMSO, cells were harvested at 96 h and were measured using Annexin V-FITC Apoptosis Detection Kit (Vazyme Biotech) according to the manufacturer's instructions. Samples were detected by FACSCAN (BD Biosciences), and data were analyzed by FlowJo V7.6.1.

Pharmacokinetic Study in Mouse

Compound dissolved in 1% Tween80/water to a concentration of 1 mg/mL, and was given to ICR mice (Male, 18-22 g, n = 3) by gavage and intravenous administration. Animal procedures were performed according to institutional ethical guidelines of animal care. Blood samples were collected at 0.25, 0.5, 1, 2, 4, 8, and 24 h after administration. 100 μL of solvent of methanol: acetonitrile (1:1, v/v) with internal standard was added to 10 μL of plasma and vortexed thoroughly. It was centrifuged for 5 min, then 20 μL of the supernatant was mixed with 20 μL of water for analysis.

Samples were analyzed by Xevo TQ-S triple quadrupole mass spectrometer. The ACQUITY UPLC BEH C18 (1.7 mm, 2.0 mm × 50 mm, Waters, USA) was used for the analysis. Gradient elution was applied consisting of 5 mM ammonium acetate aqueous solution containing 0.1% formic acid and acetonitrile containing 0.1% formic acid. After analyzing the concentrations of these compounds, the value of AUC_{last}, AUC_{INF_obs} and MRT_{INF_obs} was calculated from time concentration curves in each animal using Phoenix WinNonlin (CERTARA, USA). C_{max} was determined as the maximum plasma concentration, and T_{max} was the time to reach the maximum concentration.

In vivo Xenograft Study

We established MOLM13 xenografts mode by injecting MOLM13 cells (4×10^6 cells in 100 μL serum-free RPMI 1640) subcutaneously into the right flanks of 4~5 -week-old female nude mice. All experiments were performed according to the institutional ethical guidelines on animal care and approved by the Institute Animal Care and Use Committee at Shanghai Institute of Materia Medica. Tumor bearing mice were randomized into groups and started dosing when average tumor volume reached 50~100 mm^3. Compound **49** (100 mg/kg, 0.9%NaCl) was given orally daily for the number of days indicated. Tumor growth was monitored by the measurement of tumor volume using calipers twice or three times per week using the formula: TV =length × $width^2 \times 0.5$. The relative tumor volume (RTV) was calculated as follows: $RTV = Vt/V_0$, where Vt is the tumor volume at different time point and V_0 is the tumor volume at the beginning of the treatment. Tumor Growth Inhibition value (TGI) was calculated using

the formula: TGI (%) = [1 – Vt/Vc] *100%, where Vc and Vt are the tumor volume in the absence and presence of treatment. Body weight was measured twice or three times per week. Mice were euthanized, and tumor tissues were collected 6 hours after the last dosing and prepared for Western Blot.

ASSOCIATED CONTENT

Supporting information

Diffraction data and structure refinement statistics of crystallography; molecular docking result; extended data series for tested compounds.

Molecular formula strings (CSV).

Accession Codes

The atomic coordinates and experimental data have been deposited into the RCSB Protein Data Bank with accession numbers of 6IZQ.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. Zuhao Guo and Zhuqing Zhang contributed equally to this work.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

PRMT, protein arginine methyltransferase; SAM, S-5'-adenosyl-L methionine; MMA, monomethylarginine; sDMA, symmetric dimethylarginine; aDMA, asymmetric dimethylarginine; CARM1, coactivator associated arginine methyltransferase 1; SBDD, structure based drug discovery; SAR, structure−activity relationship; PKMT, protein lysine methyltransferase; EZH2, enhancer of zeste homolog 2 subunit; MLL1, mixed lineage leukemia 1; NSD2, nuclear receptor binding SET domain protein 2; SMYD3, SET and MYND domain-containing protein 3; DOT1L, disruptor of telomeric

silencing 1-like; Boc, *tert*-butyloxycarbonyl; TBS, *tert*-butyl-dimethylsilyl; DCM, dichloromethane; DMSO, dimethyl sulfoxide; THF, tetrahydrofuran; EA, ethyl acetate; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DMAP, 4-dimethylaminopyridine; *t*-BuBrett-Phos, bis(2-methyl-2-propanyl)(2',4',6'-triiso-propyl-3,6-dimethoxy-2-biphenylyl)phosphine; XPhos, dicyclohexyl(2',4',6'-tri-isopropyl-2-biphenylyl) phosphine.

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