Journal of Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.8b01077 • Publication Date (Web): 20 Sep 2018

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Structure-Based Design of 1-Heteroaryl-1,3-Propanediamine Derivatives as a Novel Series of CC-chemokine Receptor 5 (CCR5) Antagonists

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ABSTRACT:

CC-chemokine receptor 5 (CCR5) is an attractive target to prevent the entry of human immunodeficiency virus-1 (HIV-1) into human host cells. Maraviroc is the only one CCR5 antagonist marketed in 2007. To overcome the shortcomings of maraviroc, structure-based drug design was performed to minimize CYP450 inhibition, and to enhance anti-HIV potency and bioavailability. Thirty-four novel 1-heteroaryl-1,3-propanediamine derivatives (**1**-**34**) were synthesized, displaying CCR5 antagonist activities in the 2.3–296.4 nM range. Among these, compounds **21** and **34** were the most potent CCR5 antagonists, with excellent *in vitro* anti-HIV-1 activity and low cytotoxicity, an acceptable pharmacokinetic profile. Furthermore, the X-ray crystal structures of compounds **21** and **34** bound to CCR5 were determined at 2.8 Å resolution. Compound **34** exhibited no CYP450 inhibition activity at 25 μM, which overcome the potential drug-drug interaction of maraviroc. Compound **34** represents a promising drug candidate for the HIV infection treatment.

Key Words: CC-chemokine receptor 5, antagonist, HIV-1, structure-based design, X-ray crystal structure

INTRODUCTION

Despite worldwide efforts to prevent the epidemic of acquired immune deficiency syndrome (AIDS), it remains a global threat: currently, 36.7 million individuals live with HIV/AIDS. There are roughly 2.1 million new infections each year, and approximately 1.1 million AIDS-related deaths in 2015. Antiretroviral therapy (ART) delays progression to AIDS by reducing the viral load in infected individuals.[1] However, there remain many problems in current HAART, including unwanted side effects, drug-drug interactions, and viral resistance.[2] Thus, developing a new class of anti-HIV-1 agents with superior efficacy and safety profiles is required urgently. Preventing HIV entry into human host cells is an attractive way to control viral infection and replication.[3] CCR5 serves as a co-receptor for HIV-1 entry into $CD4^+$ T-cells.[4] The importance of CCR5 on HIV-1 infection was discovered by the observation that the naturally occurring mutation known as CCR5-Δ32 in the CCR5 gene provides resistance to infection.[5]

Many CCR5 allosteric antagonists have been reported as shown in Figure 1, however, very few have progressed into the clinic before studies were halted,[6-18] mostly because of challenging drug-like properties (antiviral activity, human *ether-à-go-go* related gene (hERG) activity, suitable bioavailability, and toxicity). Thus, only one CCR5 antagonist (maraviroc in 2007) has been approved for the treatment of HIV patient.[19] By systematical SAR optimizations, it was found that the cytochrome P450 (CYP450) inhibition and hERG inhibition were associated with the tropane moiety, triazole moiety, and amide moiety. Reducing the lipophilicity and

maintaining antiviral activity is very important for the CCR5 antagonist. Maraviroc was discovered and development by the optimization of binding potency against CCR5, antiretroviral activity, absorption, pharmacokinetics, and selectivity for the hERG channel. However, there are some factors that limit its wide prescription, such as CYP450 inhibition (CYP3A4-M IC_{50} = 3.1 μM), drug-drug interactions (the dose should be adjusted when administrated with CYP3A4 inhibitors or CYP3A4 inducers), and viral resistance. Therefore, with the rapid rise in the number of patients with HIV worldwide, discovering novel therapeutics to treat HIV with better efficacy and fewer side effects has been a research focus in academia and industry.

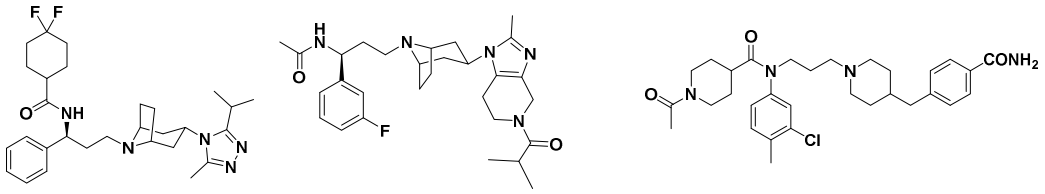


Figure 1. Representative structures of known CCR5 antagonists.

RESULT AND DISCUSSION

Design of 1-heteroaryl-1,3- propanediamine scaffold.

Maraviroc is associated with some side effects in clinical use, such as hepatotoxicity, skin hypersensitivity reaction, and cardiovascular events, which result from its shortcomings, such as potential CYP450 enzyme inhibition, drug-drug interactions, viral resistance, and high administration dose. Moreover, the physicochemical properties and pharmacokinetic profile of maraviroc need to be further optimization. In rat pharmacokinetic study, after a single oral dose of 10 mg/kg maraviroc, the maximum plasma concentration (C_{max}), the area under the

plasma concentration–time curve (AUC), and bioavailability (*F*) were 5.5 ng/mL, 12.4 ng·h/mL, and 6.0%, respectively. Metabolic studies indicated there were four primary metabolic sites/pathways for maraviroc (**M-1**–**M-4**): hydroxylation at the *para*-position of the phenyl ring (**M-1**), oxidation in the difluorocyclohexyl ring (**M-2**), oxidation of the triazole moiety (**M-3**) and *N*-dealkylation adjacent to the tropane ring (**M-4**). Accordingly, we aimed to block these metabolism sites to improve the metabolic stability, and thus increase the oral bioavailability of maraviroc.

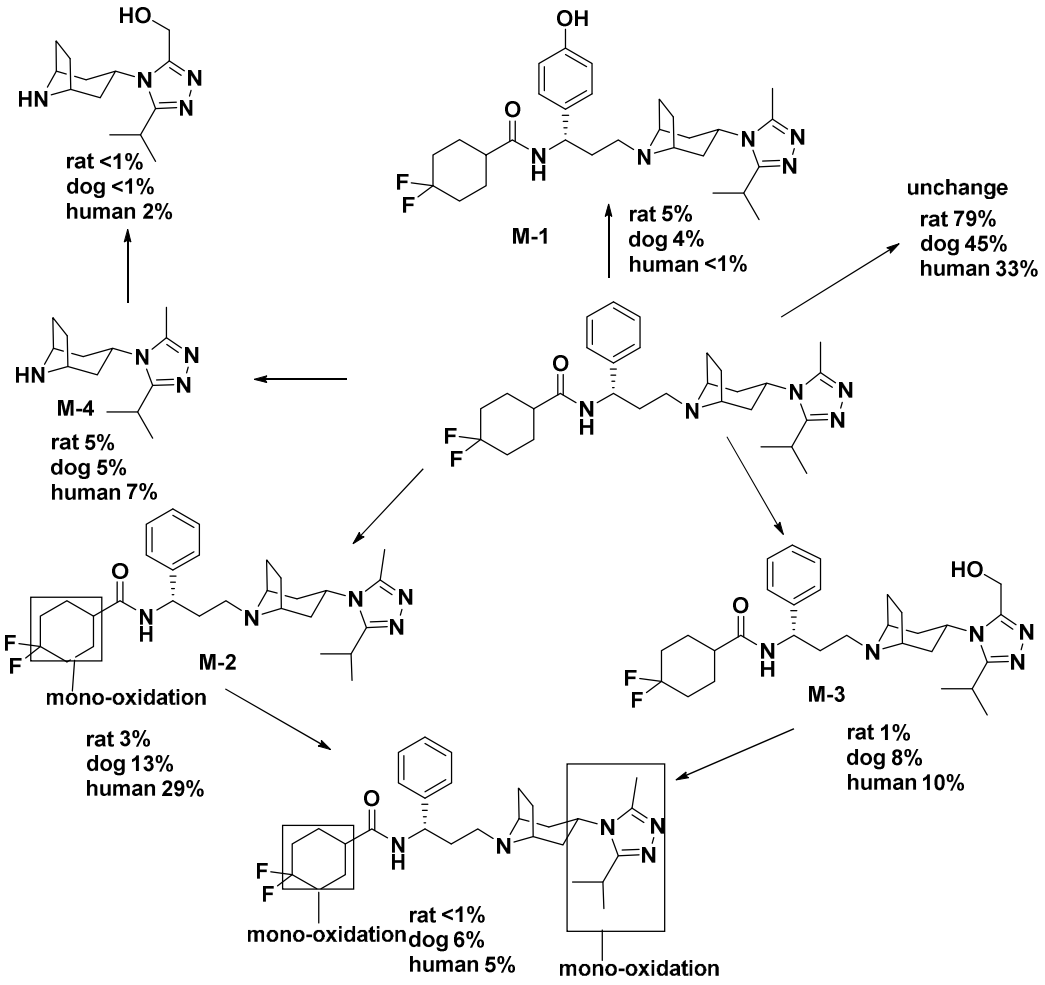


Figure 2. Metabolic pathways of maraviroc in rat, dog, and human.

Recently, Wu *et al.* reported the structure of the CCR5-maraviroc complex (Figure 3a).[20] This work reveals that maraviroc binds to a deep pocket formed by residues of the transmembrane helices and this binding site does not overlap with the major recognition site for chemokines, suggesting that maraviroc stabilizes CCR5 in an inactive state to inhibit the receptor's function *via* an allosteric mode of action. The interactions between maraviroc and CCR5 involve four distinct regions (Figure 3b). (1) P1: The fluorines in the cyclohexane ring form two hydrogen bonds with Thr-195 and Thr-259, the carboxamide nitrogen forms a hydrogen bond with Tyr-251, and the cyclohexane group makes hydrophobic contacts with a CCR5 subpocket. (2) P2: The phenyl group reaches deep into a pocket, and forms hydrophobic interactions with five aromatic residues (Tyr-108, Phe-109, Phe-112, Trp-248, and Tyr-251), especially the π-interaction with the conserved aromatic residue Trp-248, preventing the residue from moving to an active state conformation. (3) The linker: tropane moiety. The protonated nitrogen is engaged in a salt-bridge interaction with Glu-283, and the length of the carbon chain between the above-mentioned two nitrogens was reported to be critical to maintain those main forces.[21] (4) P4: The triazole group forms a hydrogen bond with Tyr-37 *via* a water molecule. Based on the crystal structure of the maraviroc with CCR5, the amide moiety ("head" part) and triazole moiety ("tail" part) are very important for the CCR5 binding activity. In addition, analyzing the metabolic pathways of the maraviroc, the phenyl group, amide moiety and triazole moiety are the major metabolic sites. In order to improve the CCR5 binding affinity and

increased the metabolic stability, a novel series of 1-heteroaryl-1,3-propanediamine derivatives (**1-34**) were designed (Figure 4).

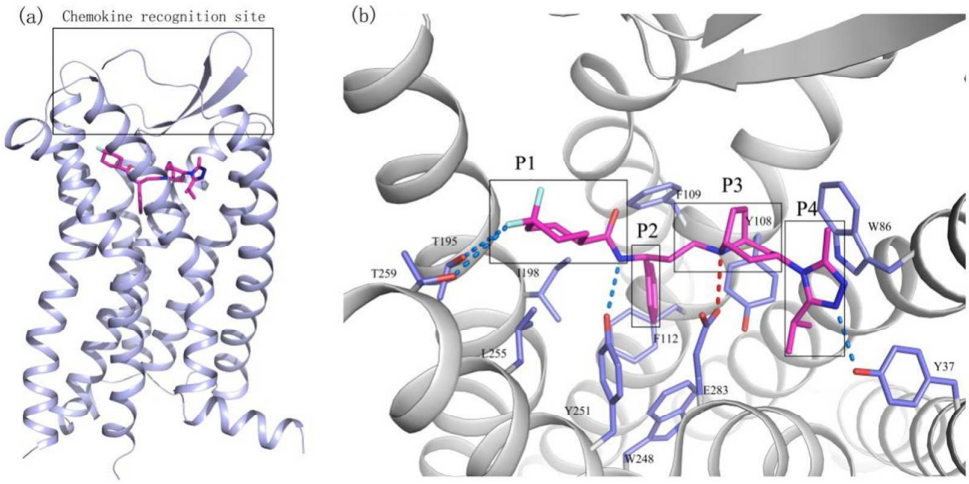


Figure 3. (a): CCR5 (PDB ID: 4MBS, light blue)/maraviroc (magenta carbons) complex structure. Maraviroc is shown in magenta stick representation. The area in black frame is known as the recognition site for chemokine. (b): CCR5 (gray) binding pocket for maraviroc (magenta carbons). Some key interactions are shown as blue (hydrogen bond) and red (salt bridge) dashed lines. Black frames highlight four distinct positions of maraviroc (P1-P4).

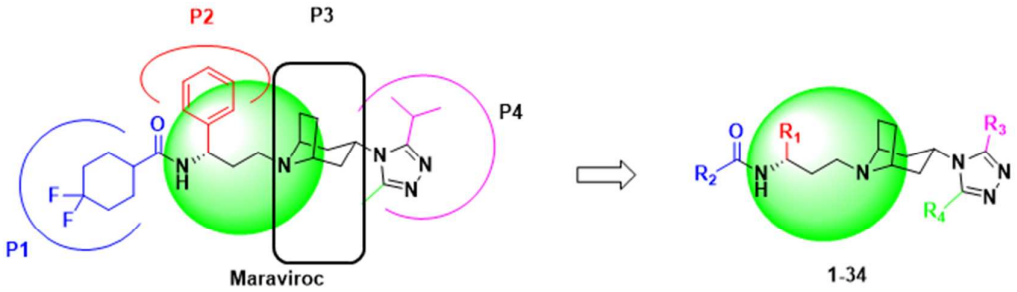
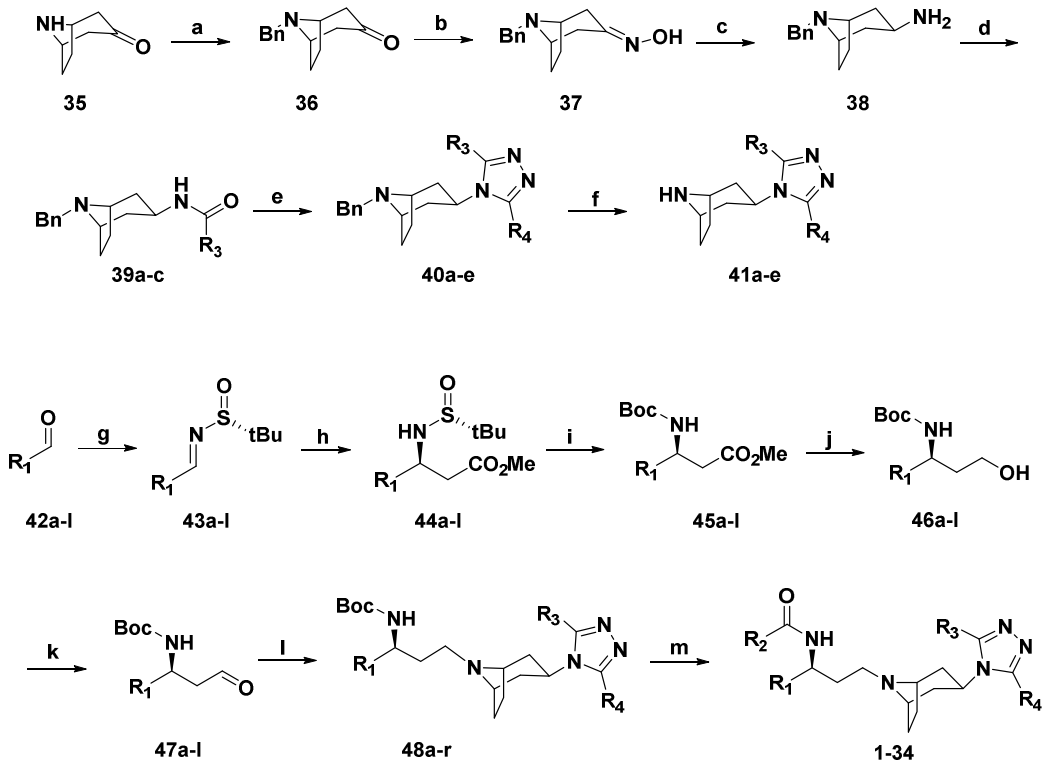


Figure 4. Design of 1-heteroaryl-1,3-propanediamine scaffold.

Chemistry.

The general synthetic route to compounds **1-34** is shown in Scheme 1. Condensation following protection of ketone **35** provided intermediate oxime **37**. The intermediate **38** was obtained by the sodium metal reduction of **37**, and acetylation of **38** provided intermediate amides **39a-c**. The triazoles **40a-e** were synthesized in three steps: activation of **39a-c** to their corresponding imidoyl chlorides, followed by trapping with hydrazides, and acid catalyzed cyclization to the triazoles. Deprotection of compounds **40a-e** provided key intermediate compounds **41a-e**. The chiral *β*-amino acids **45a-l** were prepared in three steps from aldehydes **42a-l** reacting with Ellman's chiral auxiliary. Oxidation following reduction of **45a-l** provided intermediates **47a-l**, which further reacted with **41a-e** producing compounds **48a-r**. Deprotection of compounds **48a-r**, and introduction of a range of acyl groups provided the final compounds **1-34**.

Scheme 1. Synthetic routes to thedesired compounds 1-34[a]



[a] Reagents and conditions: (a) BnBr, K_2CO_3, MeCN; (b) $NH_2OH \cdot HCl$, K_2CO_3, EtOH; (c) Na, *n*-$C_5H_{11}OH$, 100°C; (d) R_3COCl, Na_2CO_3, DCM; (e) (1) PCl_5, $R_4CONHNH_2$, DCM; (2) AcOH, 1,4-dioxane, 80°C; (f) 10% Pd/C, $HCOONH_4$, EtOH; (g) (*R*)-(+)-2-Methyl-2-propanesulfinamide, $Ti(EtO)_4$, THF; (h) LDA, CH_3COOMe, (*i*-PrO)$_3$TiCl, THF; (i)(1) 2*N* HCl, MeOH; (2) $(Boc)_2O$, NEt_3, MeOH; (j) $NaBH_4$, THF, reflux; (k) Dess-Martin periodinane, DCM; (l) **41a-e**, $NaBH(OAc)_3$, AcOH, DCE; (m) (1) 2 *N* HCl, MeOH; (2) R_2COOH, EDCI, HOBt, NEt_3, DCM.

Structure-Activity Relationships of 1-heteroaryl-1,3-propanediamine Derivatives.

Initially, all 1-heteroaryl-1,3-propanediamine derivatives (**1-34**) were evaluated their inhibitory effects on the CCR5 binding activities in the HEK293 cells with maraviroc as the positive control. As shown in Table 1, most of the compounds

generally showed potent inhibitory activity against CCR5. Firstly, different substituted heterocyclic groups were investigated at the R_1. Compound **1** containing the 2-thienyl group showed excellent activity toward CCR5 (IC_{50} = 9.1 nM). Replacement of the 2-thienyl group of compound **1** to 4-thiazolyl (compound **2**) and 3-pyridinyl (compound **3**) resulted in the decreased inhibitory activities. When the 2-thienyl group was replaced by 2-fluoro-5-pyridinyl, compound **4** showed significantly decreased the potency (IC_{50} = 251.6 nM). However, introducing the bulky 3-benzothienyl group, compound **5** maintained the potent antagonism of CCR5, with an IC_{50} value of 9.7 nM, indicating that large group could be tolerated at R_1. Then, introduction of different substituted group at the 2-thienyl group, compounds **6-11** bound to CCR5 with IC_{50} values of 2.8, 6.8, 15.8, 12.0, 8.1, and 6.8 nM, respectively. Compounds **6**, **7**, and **10** were slightly more potent than that of maraviroc.

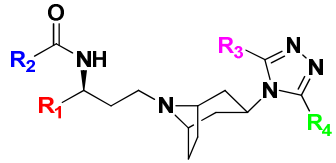
Second, we turned the structure modification to the "head" carboxamide group, the carboxamide N1 forms a hydrogen bond with Tyr-251, and cyclohexane group makes hydrophobic contacts with the CCR5 subpocket. To probe the structure−activity relationships at this site, compounds (**12-18**) were designed and synthesized with different carboxamide groups, and their binding affinities to CCR5 were determined. Replacement the 4,4-difluorocyclohexyl group with a phenyl group, 4-fluorobenzyl group, 4-acetylpiperidinyl group, 4-methylpiperidinyl group, 4-tetrahydro-2*H*-pyranyl group, cyclopentyl group or isopropyl group afforded compounds **12-18**. Among them, compound **17** was the most active CCR5 antagonist (IC_{50} = 2.6 nM), which was about 3-fold more potent than that of maraviroc (IC_{50} = 8.0 nM).

Then, replacing the 4,4-difluorocyclohexyl group with a 4-fluorocyclohexyl group, cyclohexyl group, cyclopentyl group, 1-trifluoromethyl-1,1-dimethyl group,

3-oxocyclopentyl group, 5-oxopyrrolidine group, 1-methyl-5-oxopyrrolidine group, 3,3-difluorocyclopentyl group or 3,3-difluorocyclobutyl group yielded compounds **19-27**. The 2-thienyl substituted derivatives (**19-27)** presented more potent inhibitory effects against CCR5 than the 5-methoxythienyl derivatives (**12-18**). Among them, six compounds (**19**, **20**, **21**, **23**, **26**, and **27**) exhibited significant inhibitory potency against CCR5, which was approximately more than 2-fold higher than that of maraviroc (IC_{50} = 8.0 nM).

We further investigated the effect of the "tail" triazole group. Finally, to block the metabolic sites, replacing isopropyl with cyclopropyl or ethyl afforded compounds **28-30**, Compounds **28-30** displayed potent CCR5 inhibitory activity, with IC_{50} values of 2.6, 2.9, and 3.0 nM, respectively. These results indicated that the cyclopropyl moiety could improve the activity. Replacement the methyl group with a trifluoromethyl group yielded compounds **31**, **32**, and **33**. These compounds proved to be potent CCR5 antagonists with IC_{50} values below 5.0 nM. Replacing R_1 phenyl group to the 3-thienyl group afforded compound **34** (IC_{50} = 3.0 nM), which exhibited2-fold more potent than maraviroc (IC_{50} = 8.0 nM) for antagonizing CCR5.

Table 1. CCR5 binding activity of 1-heteroaryl-1,3-propanediamine derivatives



Compd.	R_1	R_2	R_3	R_4	CCR5 antagonist activity IC_{50} (nM) [a]	cLog *P*
1	thiophen-2-yl	4,4-difluoro-cyclohexan-1-yl	methyl	isopropyl	9.1±3.0	3.37
2	thiazol-4-yl	4,4-difluoro-cyclohexan-1-yl	methyl	isopropyl	27.1±6.8	2.07
3	pyridin-3-yl	4,4-difluoro-cyclohexan-1-yl	methyl	isopropyl	82.6±20.8	1.77
4	6-fluoropyridin-3-yl	4,4-difluoro-cyclohexan-1-yl	methyl	isopropyl	251.6±57.0	1.99
5	benzo[b]thiophen-3-yl	4,4-difluoro-cyclohexan-1-yl	methyl	isopropyl	9.7±1.6	4.75
6	5-methylthiophen-2-yl	4,4-difluoro-cyclohexan-1-yl	methyl	isopropyl	2.8±1.4	3.87
7	4-methylthiophen-2-yl	4,4-difluoro-cyclohexan-1-yl	methyl	isopropyl	6.8±3.2	3.82

8	5-fluorothiophen-2-yl	4,4-difluoro-cyclohexan-1-yl	methyl	isopropyl	15.8±3.4	3.55
9	5-chlorothiophen-2-y	4,4-difluoro-cyclohexan-1-yl	methyl	isopropyl	12.0±1.2	4,74
10	4-methylthiophen-3-yl	4,4-difluoro-cyclohexan-1-yl	methyl	isopropyl	8.1±0.9	3.87
11	5-methoxythiophen-2-yl	4,4-difluoro-cyclohexan-1-yl	methyl	isopropyl	6.8±1.4	3.56
12	5-methylthiophen-2-yl	phenyl	methyl	isopropyl	51.7±7.2	4.20
13	5-methylthiophen-2-yl	4-fluorobenzyl	methyl	isopropyl	33.0±9.2	4.33
14	5-methylthiophen-2-yl	1-acetylpiperidin-4-yl	methyl	isopropyl	87.9±21.6	1.11
15	5-methylthiophen-2-yl	1-methylpiperidin-4-yl	methyl	isopropyl	296.4±47.6	1.71
16	5-methylthiophen-2-yl	tetrahydro-2H-pyran-4-yl	methyl	isopropyl	24.5±4.6	2.08
17	5-methylthiophen-2-yl	cyclopentyl	methyl	isopropyl	2.6±0.1	3,92
18	5-methylthiophen-2-yl	*i*-Pr	methyl	isopropyl	16.2±3.1	3.38
19	thiophen-2-yl	4-fluorocyclohexyl	methyl	isopropyl	3.0±0.4	3.43
20	thiophen-2-yl	cyclohexyl	methyl	isopropyl	2.3±0.3	3,98

21	thiophen-2-yl	cyclopentyl	methyl	isopropyl	3.1±0.2	3.42
22	thiophen-2-yl	2,2,2-trifluoro-1,1-dimethylethyl	methyl	isopropyl	5.4±2.0	3.36
23	thiophen-2-yl	3-oxocyclopentyl	methyl	isopropyl	2.9±0.4	1.87
24	thiophen-2-yl	(*S*)-5-oxopyrrolidin-2-yl	methyl	isopropyl	20.6±3.7	1.19
25	thiophen-2-yl	1-methyl-5-oxopyrrolidin-3-yl	methyl	isopropyl	5.2±2.0	2.40
26	thiophen-2-yl	3,3-difluorocyclopentyl	methyl	isopropyl	2.3±0.2	2.81
27	thiophen-2-yl	3,3-difluoro-cyclobutane -1-yl	methyl	isopropyl	2.6±0.2	2.25
28	thiophen-3-yl	4,4-difluoro-cyclohexan-1-yl	methyl	cyclopropyl	2.6±0.2	2.88
29	thiophen-2-yl	4,4-difluoro-cyclohexan-1-yl	methyl	cyclopropyl	2.9±0.1	2.88
30	thiophen-3-yl	4,4-difluoro-cyclohexan-1-yl	methyl	ethyl	2.8±0.1	2,97
31	5-methylthiophen-2-yl	4,4-difluoro-cyclohexan-1-yl	trifluoromethyl	isopropyl	4.0±1.1	3.97
32	thiophen-2-yl	4,4-difluoro-cyclohexan-1-yl	trifluoromethyl	isopropyl	4.7±0.8	4.37
33	thiophen-3-yl	4,4-difluoro-cyclohexan-1-yl	trifluoromethyl	cyclopropyl	2.9±0.1	2.88

34	thiophen-3-yl	4,4-difluoro-cyclohexan-1-yl	methyl	isopropyl	3.0 ± 0.3	3.37
MVC		-			8.0 ± 2.7	3.26

[a] IC_{50} values are exhibited by means ± standard deviations, $n \geqslant 3$.

Anti-HIV-1 Activity, and the Cellular Cytotoxicity of Compounds of 1-heteroaryl-1,3-propanediamine Derivatives.

Based on the CCR5 binding results, some high binding affinity CCR5 antagonists were selected for further screening *in vitro* properties. The data from an evaluation in an antiviral assay using TZM-bl cells (a frequently used HIV-1 reporter cell line) from lab-adapted HIV-1_{SF162} are shown in Table 2. All compounds showed potent anti-HIV-1 activity: the IC_{50} values of compounds **1**, **6**, **20**, **21**, and **34** were 3.6, 11.5, 11.1, 1.3, and 1.5 nM, respectively. The TI (therapeutic index) values of compounds **1**, **6**, **20**, **21**, and **34** were more than 5.5×10^4, 1.7×10^4, 1.8×10^4, 1.5×10^4, and 1.3×10^4 respectively. Compounds **1, 21,** and **34** possessed 2.3-, 6.6-, and 4.5-fold enhanced antiviral activity, respectively, compared to that of maraviroc. It is worth noting the similarity in cLog*P* (3.37, 3.87, 3.98, 3.42 and 3.37 for **1**, **6**, **20**, **21**, and **34**), which displayed excellent *in vitro* anti-HIV-1_{SF162} activity with high therapeutic index (TI) values.

Table 2. Anti-HIV-1 Activity, Cellular Cytotoxicity of 1-heteroaryl-1,3-propanediamine Derivatives

Compd.	TZM-bl IC_{50} (nM) [a]	TZM-bl CC_{50} (μM) [b]	TI [c]
1	3.6±0.3	>200	$>5.5\times10^4$
3	1520.3±184.5	>100	>65.8
5	810.5±299.5	96.1	118.6

6	11.5±0.5	>200	$>1.7\times10^4$
8	519.5±127.5	>200	>385.0
20	11.1±1.8	>200	$>1.8\times10^4$
21	1.3±0.1	>200	$>1.5\times10^4$
34	1.5±0.1	>200	$>1.3\times10^4$
MVC	6.7±0.1	>200	$>2.9\times10^4$

[a] IC_{50}s are exhibited by means ± standard deviations, n⩾3.

[b] CC_{50}s are exhibited by means ± standard deviations, n⩾3.

[c] TI, therapy index, the rate of CC_{50}/EC_{50}.

Further Assessment of anti-HIV-1 Activity, and the Cellular Cytotoxicity of compounds 1, 6, 20, 21, and 34.

To establish their broad activity spectrum, compounds **1**, **6**, **20**, **21**, and **34** wereselected for further testing as these offered a better balance of *in vitro* properties. The antiviral activity against a range of CCR5-tropic HIV-1 isolates (HIV-1_{SF162}, HIV-1_{Ba-L}, and HIV-1_{KM018}) in peripheral blood mononuclear cells (PBMC), HOS-CD4-CCR5 (human osteosarcoma (HOS) cells expressing CD-4 and CCR5) and PM-1cells (T-lymphoid cells) are shown in Table 3. Compounds **1**, **6**, **20**, **21**, and **34** were active at low nanomolar concentrations against HIV-1 with low cytotoxicity. Especially, compound **34** showed good activity against HIV-1_{SF162} in PBMCs (IC_{50} = 4.3 nM, CC_{50} = 674.8 μM, TI = 1.6×10^5), HIV-1_{Ba-L} in HOS-CD4-CCR5 (IC_{50} = 1.2 nM, CC_{50} = 337.5 μM, TI = 2.8×10^5), HIV_{Ba-L} in PM-1 (IC_{50} = 2.8 nM, CC_{50} = 735.9 μM, TI = 2.6×10^5) and HIV-1_{KM018} in PBMC (IC_{50} = 2.3 nM, CC_{50} = 674.8 μM, TI = 2.9×10^5).

Table 3. Anti-HIV-1 Activity, Cellular Cytotoxicity of Compounds **1**, **6**, **20**, **21**, and **34**

Compd.	HIV-1_{SF162}						HIV-1_{Ba-L}									HIV-1_{KM018}		
	PBMC			HOS			PBMC			HOS			PM-1			PBMC		
	IC_{50} [a] (nM)	CC_{50} [b] (nM)	TI[c]	IC_{50} [a] (nM)	CC_{50} [b] (nM)	TI[c]	IC_{50} [a] (nM)	CC_{50} [b] (nM)	TI[c]	IC_{50} [a] (nM)	CC_{50} [b] (nM)	TI[c]	IC_{50} [a] (nM)	CC_{50} [b] (nM)	TI[c]	IC_{50} [a] (nM)	CC_{50} [b] (nM)	TI[c]
1	2.9±1.3	268.0±9.7	9.2×10^4	13.1±0.8	369.7±11.0	2.8×10^4	28.5±8.8	268.0±9.7	9.4×10^3	3.6±2.4	369.7±11.0	1.0×10^5	6.2±0.8	617.5±49.6	9.9×10^4	4.8±1.1	268.0±9.7	5.6×10^4
6	6.7±1.6	>200	$>2.9\times10^4$	18.8±1.4	>200	$>1.1\times10^4$	240±4.3	>200	>833.3	11.4±1.7	>200	$>1.8\times10^4$	14.9±7.9	>200	$>1.3\times10^3$	126.2±23.0	>200	$>1.6\times10^3$
20	524.2±69.0	266.7±12.7	508.8	43.7±7.4	342.9±12.7	7.8×10^3	463.9±99.3	266.7±12.7	575.1	12.5±2.4	342.9±12.7	2.7×10^4	8.2±2.9	360.7±15.9	4.4×10^4	504.5±58.5	266.7±12.7	528.8
21	123.4±30.6	755.0±37.4	6.1×10^3	21.0±2.8	525.42±8.94	2.5×10^4	127.4±24.9	755.0±37.4	5.9×10^3	1.6±0.3	525.4±8.9	3.3×10^5	3.4±0.7	758.4±6.5	2.2×10^5	90.0±33.0	755.0±37.4	2.0×10^3
34	4.3±1.6	674.8±6.9	1.6×10^5	4.9±4.3	337.5±24.3	6.9×10^4	3.0±1.8	674.8±6.9	2.2×10^4	1.2±0.4	337.5±24.3	2.8×10^5	2.8±0.3	735.9±4.4	2.6×10^5	2.3±0.1	674.8±6.9	2.9×10^5
MVC	1.0±0.5	>200	$>2.0\times10^5.0$	4.7±0.4	>200	$>4.3\times10^4$	12.3±2.9	>200	$>1.6\times10^4$	1.9±0.7	>200	$>1.1\times10^5$	1.0±0.1	>200	$>2.0\times10^5$	2.0±0.5	>200	$>1.0\times10^5$

[a] EC_{50}s are exhibited by means ± standard deviations, n≥3.

[b] CC_{50}s are exhibited by means ± standard deviations, n≥3.

[c] TI, therapy index, the rate of CC_{50}/EC_{50}.

Determination of Co-crystal Structure of CCR5 Bound to Compounds 21 and 34.

To understand the structural basis for the high binding affinity of this series of compound to CCR5, we determined the crystal structure of CCR5 in complex with **21** and **34** at 2.8 Å resolution respectively (Figure 5). In the CCR5-**21** structure (Figure 5a), the binding model indicates that compound **21** interact with CCR5 in a similar way to the maraviroc: the thiophene moiety reaches deep into the binding pocket, forming hydrophobic interactions with five aromatic residues: Tyr-108, Phe-109, Phe-112, Trp-248, and Tyr-251, which stabilize CCR5 in an inactive conformation. The protonated nitrogen of the tropane moiety is engaged in a salt-bridge interaction with Glu-283. The triazole group forms a hydrogen bond with Tyr-371 via a water molecule. The carboxamide nitrogen forms a hydrogen bond with Tyr-251, and the cyclopentane group makes hydrophobic contacts with CCR5. In the CCR5-**34** complex structure (Figure 5b), the *meta*-position sulphur on thiophene is buried deeper into the binding pocket than the sulphur at the *ortho*-position in **34**. In addition to the polar interactions between the ligand and the residues Glu-283, Tyr-37, and Tyr-251 in the receptor, another two hydrogen bonds are formed by one of the fluorines in the cyclohexane ring of compound **34** with Thr-195 and Thr-259 of CCR5.

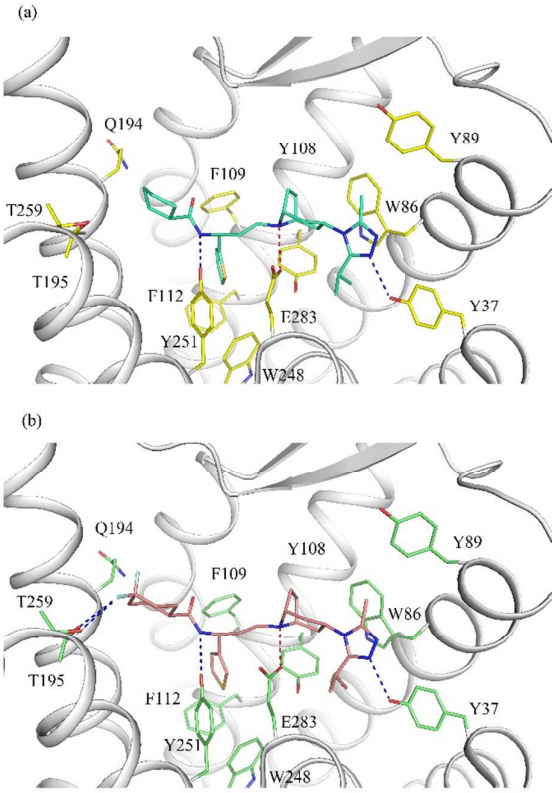


Figure 5. (a) Compound **21** (PDB ID: 6AKX), (b) Compound **34** (PDB ID: 6AKY), and receptor residues involved in ligand binding are shown in stick representation. Some key interactions are shown as blue dashed lines.

Pharmacokinetic (PK) Profiles of the Selected CCR5 Antagonists.

It was reported that maraviroc has poor bioavailability following oral administration in rats. To gain an initial understanding of the pharmacokinetic parameters of our series, we selected compounds **1**, **6**, **20**, **21**, and **34**, which had potent antiviral activities, as representatives for further investigation following intravenous (*i.v.*) and oral (*p.o.*) dosing in rats. The results (Table 4) showed that all compounds had better bioavailability compared with maraviroc. Compound **6** showed a high maximal concentration (C_{max} = 580.2 ng/mL), a much longer half-life ($t_{1/2}$ = 32.8 h) and a much

better oral bioavailability (40.1% *vs* 6.0%). Compound **20** showed a high maximal concentration (C_{max} = 631.3 ng/mL), a low half-life ($t_{1/2}$ = 1.16 h) and an acceptable oral bioavailability (F = 14.8%). Compounds **21** and **34** showed high maximal concentration (C_{max} = 436.3 ng/mL and 306.4 ng/mL), appropriate half-life ($t_{1/2}$ = 1.65 h and 1.6 h) and much better oral bioavailability (33.4%, 14.5%, *vs* 6.0%). These results suggested that this series of compounds could serve as possible lead compounds for the development of CCR5 antagonists.

Table 4. Pharmacokinetic Parameters of Compounds **1**, **6**, **20**, **21**, and **34** in Rats

compd.	admin.	dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng·h/mL)	MRT (h)	Cl (L/h/kg)	F (%)
1	*p.o.*	20	266.2	4	2.1	1108	3.3	-	7.8
	i.v.	10	6841	0.25	0.77	7053	0.7	1.42	-
6	*p.o.*	20	580.2	2	32.8	1870.7	4.8	-	40.1
	i.v.	10	1692.5	0.25	1.60	2330.2	2.3	4.3	-
20	*p.o.*	20	631.3	0.25	1.16	1512.3	2.1	-	14.8
	i.v.	10	4316.7	0.25	0.31	5114.9	0.8	1.96	-
21	*p.o.*	20	436.3	2	1.65	1671.8	3.02	-	33.4
	i.v.	10	2221.7	0.25	1.31	2504.6	0.91	3.99	-
34	*p.o.*	20	306.4	2	1.60	1259	2.6	-	14.5
	i.v.	10	3778	0.25	1.02	4162	0.8	1.0	-
MVC[a]	*p.o.*	10	5.5	2		12.4			6.0

i.v.	1	0.9		74	-	

[a] Ref 23.

Compounds **21** and **34** were selected for further investigation following intravenous (*i.v.*) and oral (*p.o.*) dosing in dogs since it had the good results of anti-HIV activity and pharmacokinetic parameters in rats. The results (Table 5) showed that compounds **21** and **34** had lower clearance (2.8, 0.3, vs 21 L/h/kg), and compound **21** displayed equivalent oral bioavailability (38.1% *vs* 40.0%) compared with maraviroc, suggesting that it would have acceptable pharmacokinetic properties in humans.

Table 5. Pharmacokinetic Properties of Compounds **21** and **34** in Dogs

compd.	admin.	dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{0-\infty}$ (ng·h/mL)	MRT (h)	Cl (L/h/kg)	F_{po} (%)
21	p.o.	15	1155	0.8	2.5	2157	2.4	-	38.1
	i.v.	3	-	-	0.9	1100	0.7	2.8	-
34	*p.o.*	15	4613	0.8	3.9	5520	2.1	-	10.0
	i.v.	3	-	-	1.7	10889	1.6	0.3	
MVC[a]	p.o.	2	256	0.75	-	583	-	-	40.0
	i.v.	0.5	-	-	2.3	-	-	21	-

[a] Ref 23.

Metabolic pathways of compound 34 in different species liver cells.

High levels of unchanged drug in the liver cells of all species (>99% human, >98% monkey, >97% dog, >97% rat, and >97% mouse). The major metabolic pathways

were defluorination (**M1**) and oxidation (**M2-M8**) of the difluorocyclohexyl moiety, oxidation hydrogenation of the left moiety (**M9**), and oxidation of the triazole moiety (**M10-M12**) (Figure 6). According to the metabolic pathways of compound **34** in different species liver cell, the unchanged drug level is much higher than maraviroc, which improved the metabolic stability and increased bioavailability.

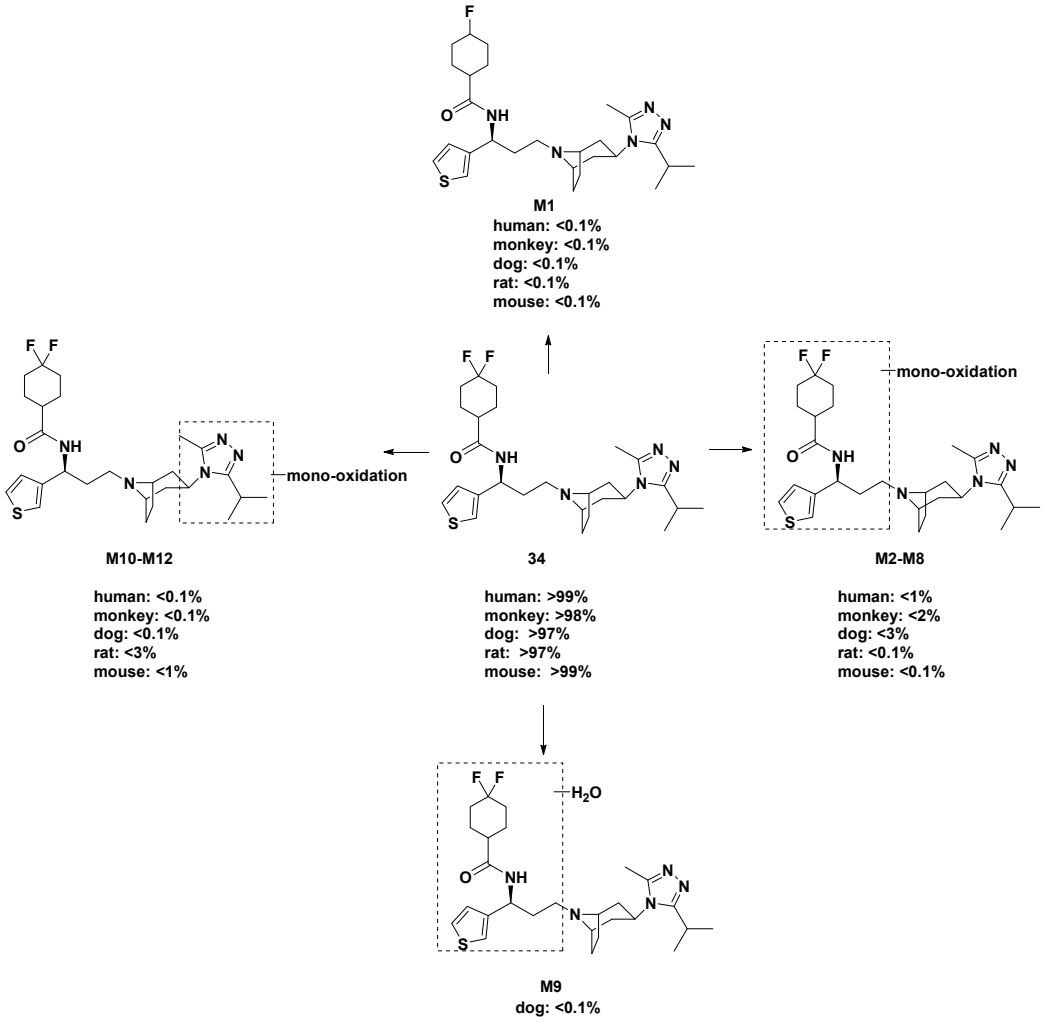


Figure 6. Metabolic pathways of compound **34** in human, monkey, dog, rat, and mouse with percentage of liver cell.

hERG and liver metabolic enzymes P450 tests of compounds 1, 6, 20, 21, and 34.

Blockade of the hERG channel is a significant hurdle encountered in drug discovery.

Compounds **1**, **6**, **20**, **21**, and **34** were selected for hERG testing (Table 6). The result showed that the IC_{50} values of compounds **1**, **6**, **20**, **21**, and **34** on hERG were 3.50, 3.38, 2.06, 1.52 and more than 40 μM, respectively. Compound **34** showed low hERG potassium ion channel inhibition.

In addition, compounds **1** and **34** were found to show no significant inhibition of any of the major P450 isoforms tested (1A2, 2C9, 2C19, 2D6, 3A4-M, and 3A4-T)(IC_{50} > 25 μM). The inhibitory activities of liver metabolic enzyme CYP3A4-M of compounds **1**, **6**, **20**, **21**, and **34** are better than maraviroc (> 25, 12.3, > 25, 10.9, > 25, vs 3.1 μM). Compounds **20** and **21** displayed higher inhibition activity against CYP2D6 than compounds **1**, **6**, **34**, and maraviroc.

Table 6. hERG and Live Metabolic Enzymes P450 Testing of Compounds of 1, 6, 20, 21 and 34

Compd.	hERG	CYP450 IC_{50} (μM)					
	IC_{50} (μM)	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4-M	CYP3A4-T
1	3.50±0.97	>25	>25	>25	>25	>25	>25
6	3.38±0.61	>25	>25	>25	>25	10.9	23.4
20	2.06±0.76	>25	>25	>25	15.1	12.3	21.7
21	1.52±0.32	>25	>25	>25	16.8	>25	>25
34	>40	>25	>25	>25	>25	>25	>25
MVC [a]	> 1	>25	14.4	>25	>25	3.1	14.9

[a] Ref 24.

Single-Dose Toxicity of Compound 34

Single-dose toxicity studies on **34** were performed in GLP laboratories. No rat died after receiving 2000 mg/kg **34** by oral gavage in the 14-day observation period. After

oral administration of **34** to beagle dogs, the maximal tolerance dose (MTD) was higher than 1000 mg/kg. The above data indicate that **34** has very low acute toxicity.

CONCLUSIONS

In this study, we reported the structure-based design, synthesis, and evaluation of a new class of CCR5 antagonists. To overcome the shortcomings of maraviroc and based on the co-crystal structure of CCR5 in complex with maraviroc, a new series of compounds with a 1-heteroaryl-1,3-propanediamine scaffold. The most potent compound **34** bound to CCR5 with an IC_{50} values of 3.0 nM, and showed excellent *in vitro* anti-HIV-1 activity. The therapeutic index (TI) against anti-HIV-1_{SF162} infection of TZM-bl cells, PBMC cells, and HOS-CD4-CCR5 cells was 1.3×10^5, 1.6×10^5, and 6.9×10^4, respectively. The oral bioavailability of compound **34** in rats and dogs are 14.6% and 10.0%, respectively, suggesting that it would have acceptable pharmacokinetic properties in humans. Furthermore, the X-ray crystal structure of CCR5 bound to compounds **21** and **34** shows that it forms strong interactions with CCR5. Compound **34** displayed no inhibition with hERG at 40 μM and CYP450 at 25 μM. In summary, compound **34** holds great promise as a more potent and a safer HIV agent that might have the following advantages over maraviroc, the CCR5 inhibition activity of compound **34** is better than maraviroc, and no CYP3A4 inhibitory activity at 25 μM. Further preclinical trials on compound **34** are currently being conducted in our laboratories.

EXPERIMENTAL SECTION

Chemistry. Materials and General Methods.

The reagents (chemicals) were purchased and used without further purification. Nuclear magnetic resonance (NMR) spectroscopy was performed on a Bruker AMX-400 and AMX-300 NMR (IS as TMS). Chemical shifts were reported in parts per million (ppm, δ) downfield from tetramethylsilane. Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Low- and high-resolution mass spectra (LRMS and HRMS) were given with electric, electrospray, and matrix-assisted laser desorption ionization (EI, ESI, and MALDI) produced by a Finnigan MAT-95, LCQ-DECA spectrometer and IonSpec 4.7 T. HPLC analysis of all final biological testing compounds was carried out on an Agilent 1260 Series HPLC with an Agilent Extend-C18 column (150×4.6 mm, 5 μm) with three solvent systems (methanol/water, methanol/buffer (5 mM NH_4OAc, 0.1% HCOOH in water) or acetonitrile/buffer (5 mM NH_4OAc, 0.1% HCOOH in water)). Purity was determined by reversed-phase HPLC and was ⩾95% for all biological tested compounds.

4,4-difluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(thiophen-2-yl)propyl)cyclohexane-1-carboxamide (1).

To a solution of **48b** (47.2 mg, 0.1 mmol) in dichloromethane (5 mL) was added triethylamine (28 μL, 0.2 mmol), HOBt (14.8 mg, 0.11 mmol), EDCI (21.1 mg, 0.11 mmol), and 4,4-difluorocyclohexanecarboxylic acid (18 mg, 0.11 mmol). The mixture was stirred at room temperature for 12 h and quenched with 10 ml of water.

The mixture was extracted with dichloromethane. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in vacuum. The residue was purified by silica gel column (DCM/MeOH, 10/1) to afford compound **1** (29.1 mg, 56%)。^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (dd, J = 5.0, 2.9 Hz, 1H), 7.12 (ddd, J = 3.0, 1.4, 0.8 Hz, 1H), 7.01 (dd, J = 5.0, 1.3 Hz, 1H), 6.47 (d, J = 8.4 Hz, 1H), 5.25 (dd, J = 14.5, 7.2 Hz, 1H), 4.36 – 4.23 (m, 1H), 3.39 (dd, J = 13.7, 4.7 Hz, 2H), 2.98 (dt, J = 13.8, 6.9 Hz, 1H), 2.50 (s, 3H), 2.46 (t, J = 6.9 Hz, 2H), 2.23 – 1.98 (m, 12H), 1.94 – 1.59 (m, 11H), 1.37 (dd, J = 6.9, 1.7 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.16, 150.63, 142.93, 126.55, 126.13, 121.06, 77.28, 77.03, 76.78, 58.99, 58.40, 47.98, 47.64, 47.26, 42.95, 35.55, 35.37, 34.46, 32.82, 26.72, 25.92, 21.65, 13.14. ESI-MS *m/z*: 520.3 $[M + H]^+$. HRMS (ESI) cacld for $C_{27}H_{40}F_2N_5OS$ ($[M+H]^+$): 506.2843; found: 520.2855.

4,4-difluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(thiazol-4-yl)propyl)cyclohexane-1-carboxamide (2).

Compound **2** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (d, J = 1.7 Hz, 1H), 7.15 (dd, J = 9.6, 5.1 Hz, 2H), 5.29 (q, J = 7.3 Hz, 1H), 4.29 – 4.13 (m, 1H), 3.66 (s, 2H), 3.33 (d, J = 13.0 Hz, 2H), 2.93 (dt, J = 13.5, 6.8 Hz, 1H), 2.44 (s, 3H), 2.33 (d, J = 6.1 Hz, 2H), 2.19 (dd, J = 24.1, 12.3 Hz, 3H), 2.13 – 1.91 (m, 7H), 1.91 – 1.67 (m, 6H), 1.56 (d, J = 8.5 Hz, 6H), 1.29 (d, J = 6.8 Hz, 7H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.74, 159.17, 157.09, 153.51, 150.72, 122.71, 114.66, 59.04, 58.38, 48.10, 47.87, 47.39, 42.60, 35.81,

34.23, 32.81, 26.52, 26.42, 26.01, 21.60, 19.52, 13.05. ESI-MS *m/z*: 521.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{26}H_{39}F_2N_6OS$ ($[M+H]^+$): 521.2796; found: 521.3119.

4,4-difluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(pyridin-3-yl)propyl)cyclohexane-1-carboxamide (3).

Compound **3** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.55 (d, J = 1.9 Hz, 1H), 8.49 (dd, J = 4.7, 1.3 Hz, 1H), 7.61 (d, J = 7.9 Hz, 1H), 7.28 – 7.24 (m, 2H), 7.19 (s, 1H), 5.14 (q, J = 7.1 Hz, 1H), 4.30 (ddd, J = 13.5, 11.5, 6.2 Hz, 1H), 3.43 (d, J = 15.8 Hz, 2H), 3.02 – 2.93 (m, 1H), 2.51 (d, J = 10.2 Hz, 5H), 2.26 (dt, J = 22.0, 11.8 Hz, 4H), 2.18 – 1.97 (m, 6H), 1.95 – 1.60 (m, 10H), 1.36 (dd, J = 6.8, 2.6 Hz, 6H), 0.89 (dt, J = 14.4, 7.2 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.87, 159.19, 150.59, 148.75, 148.17, 137.64, 134.28, 123.65, 68.18, 59.21, 58.25, 50.16, 47.74, 47.11, 42.72, 38.72, 35.97, 35.22, 34.09, 33.13, 32.79, 30.36, 26.66, 25.94, 23.74, 23.00, 21.66, 13.21, 10.98. ESI-MS *m/z*: 515.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{28}H_{41}F_2N_6O$ ($[M+H]^+$): 515.32323; found: 515.3265.

4,4-difluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(4-fluoropyridin-3-yl)propyl)cyclohexane-1-carboxamide (4).

Compound **4** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 – 8.03 (m, 1H), 7.79 (t, J = 7.9 Hz, 1H), 7.71 (s, 1H), 7.19 – 7.12 (m, 1H), 5.25 (dd, J = 14.0, 8.1 Hz, 1H), 4.37 – 4.25 (m, 1H), 3.53

(s, 2H), 3.02 (dt, *J* = 13.5, 6.7 Hz, 1H), 2.61 (s, 1H), 2.54 (s, 3H), 2.36 (dd, *J* = 24.0, 12.7 Hz, 3H), 2.22 – 1.97 (m, 6H), 1.90 (s, 2H), 1.74 (d, *J* = 2.4 Hz, 8H), 1.35 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ174.34, 161.87, 159.97, 159.22, 150.74, 146.23, 139.21, 125.07, 124.19, 124.19, 122.68, 121.79, 120.77, 59.78, 58.96, 53.53, 48.36, 46.92, 42.32, 35.50, 33.23, 32.96, 26.18, 26.18, 21.65, 13.10. ESI-MS *m/z*: 533.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{28}H_{40}F_3N_6O$ ($[M+H]^+$): 533.3137; found: 533.2798.

4,4-difluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(benzothiophen-2-yl)propyl)cyclohexane-1-carboxamide (5).

Compound **5** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 – 7.76 (m, 2H), 7.39 – 7.29 (m, 2H), 5.54 (s, 1H), 4.75 (s, 1H), 4.37 – 4.20 (m, 1H), 3.62 (s, 2H), 3.09 – 2.98 (m, 2H), 2.71 (s, 2H), 2.49 (s, 3H), 2.38 – 1.98 (m, 6H), 1.92 (d, *J* = 12.9 Hz, 1H), 1.87 – 1.55 (m, 8H), 1.37 – 1.27 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.21, 159.20, 150.85, 140.50, 137.70, 136.70, 124.75, 124.35, 123.54, 122.77, 121.87, 120.82, 59.64, 59.18, 48.71, 46.84, 45.90, 45.16, 42.57, 35.33, 32.95, 32.67, 32.54, 29.68, 26.39, 25.71, 21.64, 13.07, 8.61. ESI-MS *m/z*: 570.3 $[M + H]^+$. HRMS (ESI) cacld for $C_{31}H_{42}F_2N_5OS$ ($[M+H]^+$): 570.3000; found: 570.2893.

4,4-difluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(5-methylthiophen-2-yl)propyl)cyclohexane-1-carboxamide (6).

Compound **6** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.72 (d, J = 3.4 Hz, 1H), 6.64 (s, 1H), 6.60 – 6.57 (m, 1H), 5.36 (q, J = 7.0 Hz, 1H), 4.35 – 4.24 (m, 1H), 3.40 (d, J = 21.0 Hz, 2H), 3.00 (dq, J = 13.7, 6.8 Hz, 1H), 2.51 (s, 5H), 2.44 (d, J = 0.7 Hz, 3H), 2.34 – 2.10 (m, 5H), 2.10 – 1.98 (m, 4H), 1.96 – 1.57 (m, 11H), 1.38 (dd, J = 6.9, 2.4 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.17, 159.14, 150.66, 143.26, 138.73, 124.93, 124.08, 122.63, 58.65, 58.13, 47.91, 47.63, 47.24, 42.75, 35.91, 35.61, 32.99, 32.80, 32.61, 26.76, 26.17, 21.66, 15.34, 13.16. ESI-MS *m/z*: 534.3 [M + H] $^{+}$. HRMS (ESI) cacld for $C_{28}H_{42}F_2N_5OS$ ($[M+H]^+$): 534.3000; found: 534.2993.

4,4-difluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(4-methylthiophen-2-yl)propyl)cyclohexane-1-carboxamide (7).

Compound **7** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.06 (s, 1H), 6.72 (d, J = 5.9 Hz, 2H), 5.31 (q, J = 7.3 Hz, 1H), 4.33 – 4.21 (m, 1H), 3.46 (d, J = 19.6 Hz, 2H), 2.97 (dt, J = 13.4, 6.7 Hz, 1H), 2.54 (s, 2H), 2.49 (s, 3H), 2.31 (dd, J = 27.9, 15.4 Hz, 2H), 2.22 (t, J = 11.5 Hz, 1H), 2.16 (s, 3H), 2.07 (dd, J = 14.0, 8.1 Hz, 6H), 1.87 (d, J = 8.9 Hz, 2H), 1.79 (dd, J = 16.3, 8.8 Hz, 2H), 1.75 – 1.57 (m, 6H), 1.33 (dd, J = 6.9, 3.6 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ173.63, 159.14, 150.74, 145.53, 137.52, 126.59, 122.68, 119.21, 58.96, 58.44, 48.08, 47.26, 47.09, 42.50, 35.05, 32.72, 26.41, 25.76, 21.58, 15.73, 13.02. ESI-MS *m/z*: 534.3 [M + H] $^{+}$. HRMS (ESI) cacld for $C_{28}H_{42}F_2N_5OS$ ($[M+H]^+$): 534.3000; found: 534.2965.

4,4-difluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(4-fluorothiophen-2-yl)propyl)cyclohexane-1-carboxamide (8).

Compound **8** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.27 (s, 1H), 6.48 (t, J = 3.5 Hz, 1H), 6.21 (dd, J = 3.9, 1.4 Hz, 1H), 5.16 (q, J = 6.5 Hz, 1H), 4.30 – 4.19 (m, 1H), 3.45 (d, J = 17.2 Hz, 3H), 2.95 (dt, J = 13.6, 6.8 Hz, 1H), 2.53 (s, 2H), 2.46 (s, 3H), 2.30 (d, J = 12.3 Hz, 2H), 2.22 (t, J = 10.7 Hz, 1H), 2.15 – 1.93 (m, 6H), 1.84 (s, 2H), 1.80 – 1.71 (m, 2H), 1.64 (d, J = 12.3 Hz, 6H), 1.29 (dd, J = 6.9, 3.8 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.92, 165.06, 163.15, 159.16, 150.70, 133.95, 120.27, 106.47, 59.14, 58.43, 48.15, 47.35, 47.03, 42.41, 35.45, 33.92, 32.71, 32.71, 32.71, 26.07, 25.71, 21.58, 13.04. ESI-MS *m/z*: 538.3 $[M + H]^+$. HRMS (ESI) cacld for $C_{27}H_{39}F_3N_5OS$ ($[M+H]^+$): 538.2749; found: 538.2821.

4,4-difluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(5-chlorothiophen-2-yl)propyl)cyclohexane-1-carboxamide (9). Compound **9** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (s, 1H), 6.73 (d, J = 3.8 Hz, 1H), 6.70 (d, J = 3.8 Hz, 1H), 5.34 – 5.23 (m, 2H), 4.36 – 4.21 (m, 1H), 3.46 (d, J = 12.9 Hz, 2H), 2.99 (dt, J = 13.8, 7.0 Hz, 1H), 2.57 (s, 2H), 2.51 (s, 3H), 2.43 – 1.98 (m, 9H), 1.98 – 1.56 (m, 10H), 1.36 (dd, J = 6.8, 1.6 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.64, 159.11, 157.17, 147.65, 128.48, 125.89, 123.46, 58.84, 53.42, 47.64, 47.41, 47.00, 42.57, 35.36, 32.74, 29.65, 26.52, 25.87, 21.63, 13.12. ESI-MS

m/z: 554.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{27}H_{29}ClF_2N_5OS$ ($[M+H]^+$): 554.2454; found: 554.2467.

4,4-difluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(4-methylthiophen-3-yl)propyl)cyclohexane-1-carboxamide (10).

Compound **10** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.10 (d, J = 2.1 Hz, 1H), 6.88 (dd, J = 3.0, 0.9 Hz, 1H), 6.75 (s, 1H), 5.26 (s, 1H), 5.16 (dd, J = 14.3, 6.9 Hz, 1H), 4.33 – 4.19 (m, 1H), 3.46 (s, 2H), 2.98 (dd, J = 13.5, 6.7 Hz, 1H), 2.46 (s, 4H), 2.19 (d, J = 21.1 Hz, 5H), 2.14 – 1.95 (m, 6H), 1.81 (ddd, J = 24.7, 21.0, 15.3 Hz, 5H), 1.64 (d, J = 4.3 Hz, 6H), 1.32 (d, J = 6.8 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.48, 159.15, 150.69, 141.96, 136.52, 122.04, 121.23, 58.99, 53.49, 48.55, 47.15, 45.53, 42.63, 35.78, 32.82, 29.66, 27.20, 25.77, 25.77, 21.69, 14.35, 13.07. ESI-MS *m/z*: 534.3 $[M + H]^+$. HRMS (ESI) cacld for $C_{28}H_{42}F_2N_5OS$ ($[M+H]^+$): 534.3000; found: 534.2989.

4,4-difluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(5-methoxythiophen-3-yl)propyl)cyclohexane-1-carboxamide (11).

Compound **11** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (s, 1H), 7.04 (d, J = 3.1 Hz, 1H), 6.21 (d, J = 3.3 Hz, 1H), 5.26 (s, 1H), 5.15 – 5.04 (m, 1H), 4.37 – 4.22 (m, 1H), 3.80 (s, 3H), 3.62 (s, 2H), 3.11 – 2.92 (m, 1H), 2.61 (s, 2H), 2.54 (s, 4H), 2.15 (dd, J = 40.8, 22.5 Hz, 7H), 1.82 (dd, J = 49.0, 10.1 Hz, 11H), 1.38 – 1.27 (m, 6H). ^{13}C NMR (150 MHz, $CDCl_3$)

δ 173.74, 159.17, 155.64, 150.87, 131.85, 124.38, 122.78, 121.46, 97.85, 59.06, 57.56, 53.51, 48.43, 46.20, 42.57, 34.97, 32.80, 25.89, 25.75, 21.70, 13.22. ESI-MS *m/z*: 550.3 $[M + H]^+$. HRMS (ESI) cacld for $C_{28}H_{42}F_2N_5O_2S$ ($[M+H]^+$): 550.2949; found: 550.2667.

N-((*S*)-3-(*exo*-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(5-methylthiophen-2-yl)propyl)benzamide (12).

Compound **12** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 (d, J = 6.9 Hz, 2H), 7.66 (s, 1H), 7.43 (q, J = 7.2 Hz, 1H), 7.36 (t, J = 7.6 Hz, 2H), 6.76 (d, J = 3.2 Hz, 1H), 6.54 (d, J = 2.3 Hz, 1H), 5.49 (d, J = 6.2 Hz, 1H), 5.25 (s, 1H), 4.33 – 4.20 (m, 1H), 3.55 (s, 2H), 2.99 (dt, J = 15.0, 7.5 Hz, 1H), 2.72 (s, 2H), 2.42 (s, 3H), 2.36 (d, J = 22.2 Hz, 4H), 2.14 (dd, J = 47.1, 40.4 Hz, 4H), 1.66 (d, J = 37.1 Hz, 4H), 1.31 (dt, J = 8.3, 4.2 Hz, 7H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.56, 158.68, 150.39, 142.63, 138.35, 133.94, 131.18, 128.08, 126.73, 124.49, 123.76, 53.07, 47.61, 47.46, 46.32, 45.35, 34.22, 29.23, 25.89, 25.30, 21.20, 14.90, 12.68, 8.17. ESI-MS *m/z*: 492.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{28}H_{38}N_5OS$ ($[M+H]^+$): 492.2719; found: 492.2739.

2-(4-fluorophenyl)-*N*-((*S*)-3-(*exo*-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(5-methylthiophen-2-yl)propyl)acetamide (13).

Compound **13** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 (dd, J = 8.3, 5.5 Hz, 2H), 6.94 (t, J = 8.6 Hz, 2H), 6.66 (d, J = 3.3 Hz, 1H), 6.51 (d, J = 2.3 Hz, 1H), 5.24 (dd, J = 14.4, 7.5 Hz, 1H), 4.72 (s, 1H), 4.33 – 4.18 (m, 1H), 3.49 (s, 4H), 2.99 (dt, J = 13.6, 6.8 Hz, 1H), 2.55

(s, 2H), 2.48 (s, 3H), 2.37 (s, 5H), 2.09 (dd, J = 27.5, 6.0 Hz, 5H), 1.67 (d, J = 8.8 Hz, 4H), 1.41 – 1.24 (m, 7H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.39, 162.86, 160.91, 159.19, 150.90, 142.83, 138.74, 131.09, 130.84, 124.84, 124.12, 115.52, 115.35, 59.81, 59.12, 48.39, 47.30, 46.86, 42.51, 35.35, 34.01, 25.97, 25.71, 21.62, 15.28, 13.01. ESI-MS *m/z*: 524.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{29}H_{39}FN_5OS$ ($[M+H]^+$): 524.2781; found: 524.2792.

1-acetyl-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(5-methylthiophen-2-yl)propyl)piperidine-4-carboxamide (14).

Compound **14** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18 (s, 1H), 6.69 (dd, J = 5.4, 3.6 Hz, 1H), 6.54 (d, J = 3.2 Hz, 1H), 5.25 (d, J = 7.2 Hz, 1H), 4.49 (t, J = 12.6 Hz, 1H), 4.35 – 4.22 (m, 1H), 3.87 – 3.73 (m, 1H), 3.52 (d, J = 13.7 Hz, 2H), 3.09 – 2.95 (m, 2H), 2.57 (d, J = 10.3 Hz, 3H), 2.50 (s, 3H), 2.39 (s, 6H), 2.09 (d, J = 13.5 Hz, 4H), 2.03 (s, 3H), 1.81 (dd, J = 21.6, 10.9 Hz, 2H), 1.75 – 1.54 (m, 6H), 1.33 (dd, J = 6.8, 2.7 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.60, 168.93, 159.17, 150.80, 143.03, 138.73, 124.86, 124.07, 59.74, 58.80, 48.21, 47.05, 46.86, 45.82, 42.69, 40.96, 35.35, 34.35, 31.54, 28.88, 28.51, 26.27, 25.73, 22.61, 21.64, 21.40, 15.31, 13.11. ESI-MS *m/z*: 541.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{29}H_{45}N_6O_2S$ ($[M+H]^+$): 541.3246; found: 541.2395.

1-methyl-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(5-methylthiophen-2-yl)propyl)piperidine-4-carboxamide (15).

Compound **15** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (s, 1H), 6.71 (dd, J = 5.2, 3.3 Hz, 1H), 6.51 (d, J = 3.4 Hz, 1H), 5.23 (d, J = 7.2 Hz, 1H), 4.42 (t, J = 12.6 Hz, 1H), 4.31 – 4.22 (m, 1H), 3.86 – 3.71 (m, 1H), 3.51 (d, J = 13.3 Hz, 2H), 3.07 – 2.91 (m, 2H), 2.55 (d, J = 10.1 Hz, 3H), 2.51 (s, 3H), 2.39 (s, 6H), 2.19 (d, J = 13.1 Hz, 4H), 1.93 (s, 3H), 1.83 (dd, J = 21.1, 10.2 Hz, 2H), 1.72 – 1.51 (m, 6H), 1.31 (dd, J = 6.5, 2.7 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.50, 169.03, 158.15, 151.56, 144.34, 137.67, 123.32, 122.01, 59.54, 57.81, 47.20, 46.01, 45.81, 44.72, 42.63, 40.86, 36.55, 35.15, 33.14, 27.38, 24.51, 25.21, 26.83, 21.51, 20.94, 20.40, 16.32, 14.01. ESI-MS *m/z*: 513.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{28}H_{45}N_6OS$ ($[M+H]^+$): 513.3297; found: 517.3283.

_N_-((_S_)-3-((1_R_,3_R_,5_S_)-3-(3-isopropyl-5-methyl-4_H_-1,2,4-triazol-4-yl)-8-azabicyclo [3.2.1]octan-8-yl)-1-(5-methylthiophen-2-yl)propyl)tetrahydro-2_H_-pyran-4-carb oxamide (16).

Compound **16** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.88 (s, 1H), 6.71 (d, J = 3.4 Hz, 1H), 6.58 – 6.52 (m, 1H), 5.27 (dd, J = 14.5, 7.4 Hz, 1H), 4.36 – 4.23 (m, 1H), 4.01 – 3.90 (m, 2H), 3.53 (d, J = 16.1 Hz, 2H), 3.41 – 3.31 (m, 2H), 3.01 (dt, J = 13.5, 6.7 Hz, 1H), 2.60 (s, 2H), 2.52 (s, 3H), 2.46 – 2.33 (m, 6H), 2.13 (d, J = 8.2 Hz, 5H), 1.82 – 1.60 (m, 9H), 1.35 (dd, J = 6.8, 2.6 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.81, 159.17, 150.79, 142.99, 138.80, 124.90, 124.11, 67.19, 59.70, 58.69, 48.20, 47.28, 46.95, 42.06, 35.22, 34.34, 29.19, 26.33, 25.76, 21.65, 15.32, 13.14. ESI-MS *m/z*: 500.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{27}H_{42}N_5O_2S$ ($[M+H]^+$): 500.2981; found: 500.2985.

_N_-((_S_)-3-(_exo_-3-(3-isopropyl-5-methyl-4_H_-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(5-methylthiophen-2-yl)propyl)cyclohexane carboxamide (17).

Compound **17** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.76 (d, J = 2.5 Hz, 1H), 6.61 – 6.55 (m, 1H), 5.26 (d, J = 6.3 Hz, 1H), 4.42 – 4.28 (m, 1H), 3.69 (s, 2H), 3.07 (d, J = 3.9 Hz, 1H), 2.74 (s, 3H), 2.60 (s, 5H), 2.43 (s, 3H), 2.22 (s, 4H), 1.93 – 1.65 (m, 10H), 1.56 (d, J = 7.0 Hz, 2H), 1.38 (d, J = 6.6 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.91, 159.16, 150.89, 142.82, 138.93, 124.93, 124.13, 59.22, 48.48, 47.35, 46.14, 45.47, 35.01, 30.61, 30.25, 25.90, 21.70, 15.33, 13.26. ESI-MS *m/z*: 484.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{37}H_{42}N_5OS$ ($[M+H]^+$): 484.3032; found: 484.3092.

_N_-((_S_)-3-(_exo_-3-(3-isopropyl-5-methyl-4_H_-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(5-methylthiophen-2-yl)propyl)cyclopentane carboxamide (18).

Compound **18** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.04 (s, 1H), 6.67 (d, J = 3.3 Hz, 1H), 6.49 (d, J = 2.3 Hz, 1H), 5.21 – 5.12 (m, 1H), 4.36 – 4.23 (m, 1H), 3.64 (s, 2H), 3.02 (s, 1H), 2.68 (s, 2H), 2.50 (s, 5H), 2.34 (s, 4H), 2.16 (dd, J = 17.1, 5.9 Hz, 4H), 1.74 (dd, J = 26.9, 10.2 Hz, 4H), 1.29 (dd, J = 6.6, 4.4 Hz, 6H), 1.08 (t, J = 6.3 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.30, 158.75, 150.42, 142.51, 138.27, 124.40, 123.53, 76.96, 76.75, 76.54, 59.59, 58.83, 53.08, 47.93, 46.62, 46.20, 34.97, 34.47, 32.72, 25.41, 25.26, 21.22, 19.21, 19.07, 14.86, 12.57. ESI-MS *m/z*: 485.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{25}H_{40}N_5OS$ ($[M+H]^+$): 485.2875; found: 485.2779.

4-fluoro-_N_-((_S_)-3-((1_R_,3_R_,5_S_)-3-(3-isopropyl-5-methyl-4_H_-1,2,4-triazol-4-yl)-8-a

zabicyclo[3.2.1]octan-8-yl)-1-(thiophen-2-yl)propyl)cyclohexane-1-carboxamide (19).

Compound **19** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (dd, J = 5.3, 2.1 Hz, 1H), 6.92 (s, 2H), 5.45 – 5.33 (m, 1H), 4.77 (d, J = 48.1 Hz, 1H), 4.28 (d, J = 5.8 Hz, 1H), 3.49 (s, 2H), 3.05 – 2.90 (m, 1H), 2.57 (s, 2H), 2.34 (s, 2H), 2.12 (t, J = 31.7 Hz, 7H), 1.96 – 1.49 (m, 10H), 1.34 (dd, J = 6.7, 2.4 Hz, 7H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.52, 159.11, 150.70, 145.99, 126.90, 124.03, 88.67, 86.99, 59.39, 58.57, 47.95, 47.03, 43.88, 43.43, 35.29, 31.58, 30.03, 29.82, 26.94, 26.60, 25.72, 23.65, 21.63, 13.16. ESI-MS *m/z*: 502.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{27}H_{41}FN_5OS$ ($[M+H]^+$): 502.2938; found: 502.2965.

***N*-((*S*)-3-(*exo*-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(thiophen-2-yl)propyl)cyclohexane carboxamide (20).**

Compound **20** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 – 7.26 (m, 1H), 7.13 – 7.07 (m, 1H), 7.00 – 6.95 (m, 1H), 6.80 – 6.72 (m, 1H), 5.24 – 5.16 (m, 1H), 3.97 – 3.86 (m, 1H), 3.20 – 3.07 (m, 2H), 3.05 – 2.96 (m, 1H), 2.53 – 2.50 (m, 3H), 2.36 – 1.92 (m, 8H), 1.88 – 1.67 (m, 9H), 1.61 – 1.51 (m, 2H), 1.41 – 1.32 (m, 6H), 1.12 – 1.07 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.18, 150.75, 142.93, 126.33, 121.03, 59.23, 58.96, 48.27, 47.24, 46.77, 45.57, 35.26, 29.73, 27.02, 25.66, 21.66, 13.18. ESI-MS *m/z*: 484.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{27}H_{42}N_5OS$ ($[M+H]^+$): 484.3032; found: 484.2998.

***N*-((*S*)-3-(*exo*-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]o**

ctan-8-yl)-1-(thiophen-2-yl)propyl)cyclopentane carboxamide (21).

Compound **21** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24 – 7.18 (m, 1H), 7.01 – 6.94 (m, 2H), 6.64 (d, J = 1.4 Hz, 1H), 5.51 – 5.43 (m, 1H), 4.35 – 4.25 (m, 1H), 3.42 (s, 2H), 3.05 – 2.96 (m, 1H), 2.58 – 2.44 (m, 6H), 2.35 – 2.00 (m, 7H), 1.90 – 1.51 (m, 12H), 1.43 – 1.36 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.31, 159.16, 146.25, 126.98, 124.05, 58.79, 47.94, 47.28, 45.90, 35.79), 30.57, 30.35, 26.62, 25.92, 21.67, 13.20. ESI-MS *m/z*: 470.2 [M + H] $^{+}$. HRMS (ESI) cacld for $C_{26}H_{40}N_5OS$ ([M+H]$^{+}$): 470.2875; found: 470.2883.

3,3,3-trifluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(thiophen-2-yl)propyl)-2,2-dimethylpropanamide (22).

Compound **22** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (400 MHz, $CDCl_3$) :δ 7.22 (dd, J = 5.0, 1.2 Hz, 1H), 7.00-6.93 (m, 2H), 6.65 (d, J = 7.1 Hz, 1H), 5.52 (q, J = 7.0 Hz, 1H), 4.35 – 4.22 (m, 1H), 3.39 (d, J = 15.1 Hz, 2H), 3.06 – 2.95 (m, 1H), 2.55-2.49 (m, 5H), 2.30-2.16 (m, 2H), 2.13-2.01 (m, 4H), 1.69 – 1.57 (m, 4H), 1.42 (d, J = 4.5 Hz, 6H), 1.39 (d, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.29, 159.17, 150.76, 145.55, 128.66, 127.06, 125.85, 124.30, 124.12, 59.04, 58.40, 48.37, 48.11, 47.55, 47.24, 35.55, 35.30, 35.09, 26.74, 26.68, 25.87, 21.69, 20.00, 19.97, 19.84, 19.82, 13.15. ESI-MS *m/z*: 512.2 [M + H] $^{+}$. HRMS (ESI) cacld for $C_{25}H_{37}F_3N_5OS$ ([M+H]$^{+}$): 512.2665; found: 512.2655.

***N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo**

[3.2.1]octan-8-yl)-1-(thiophen-2-yl)propyl)-3-oxocyclopentane-1-carboxamide (23).

Compound **23** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.19 (m, 1H), 7.00-6.94 (m, 2H), 5.45 (q, *J* = 7.0 Hz, 1H), 4.39 – 4.25 (m, 1H), 3.49 (d, *J* = 10.0 Hz, 2H), 3.08 – 2.88 (m, 2H), 2.64 – 2.49 (m, 6H), 2.47 – 2.25 (m, 5H), 2.24 – 2.05 (m, 6H), 1.78 – 1.62 (m, 4H), 1.38 (dd, *J* = 6.8, 1.6 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 217.20, 217.06, 172.96, 159.18, 150.71, 145.57, 145.35, 127.08, 124.44, 124.35, 124.27, 59.46, 59.38, 58.60, 48.10, 47.74, 47.67, 47.10, 42.58, 42.51, 41.68, 41.61, 37.60, 37.46, 35.56, 35.45, 27.23, 27.19, 26.49, 25.84, 21.67, 13.15. ESI-MS *m/z*: 484.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{26}H_{38}N_5OS$ ($[M+H]^+$): 484.2742; found: 484.2738.

(*S*)-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(thiophen-2-yl)propyl)-5-oxopyrrolidine-2-carboxamide (24).

Compound **24** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (400 MHz, CD_3OD) δ 7.36 (dd, *J* = 5.1, 0.7 Hz, 1H), 7.16 (d, *J* = 3.5 Hz, 1H), 7.01 (dd, *J* = 5.0, 3.6 Hz, 1H), 5.42-5.30 (m, 1H), 4.63-4.52 (m, 1H), 4.33 (dd, *J* = 8.6, 4.5 Hz, 1H), 4.22 (d, *J* = 11.3 Hz, 2H), 3.53-3.39 (m, 1H), 3.25-3.07 (m, 2H), 2.81-2.68 (m, 2H), 2.66 – 2.53 (m, 4H), 2.52 – 2.27 (m, 5H), 2.25-2.14 (m, 4H), 2.11 – 1.93 (m, 2H), 1.36 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 180.11, 173.52, 160.32, 151.37, 143.96, 126.68, 124.65, 124.58, 61.95, 61.40, 56.54, 48.55, 46.52, 46.16, 33.90, 31.14, 29.33, 29.18, 25.16, 23.87, 23.82, 20.90, 20.87, 11.18.

ESI-MS *m/z*: 485.2 [M + H] $^{+}$. HRMS (ESI) cacld for $C_{25}H_{37}N_6O_2S$ ([M+H]$^{+}$): 485.2693; found: 485.2706.

_N_-((_S_)-3-((1_R_,3_R_,5_S_)-3-(3-isopropyl-5-methyl-4_H_-1,2,4-triazol-4-yl)-8-azabicyclo [3.2.1]octan-8-yl)-1-(thiophen-2-yl)propyl)-1-methyl-5-oxopyrrolidine-2-carboxamide (25).

Compound **25** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (400 MHz, CD_3OD) δ 7.33 (d, *J* = 5.0 Hz, 1H), 7.11 (d, *J* = 3.4 Hz, 1H), 7.04 – 6.94 (m, 1H), 5.40-5.31 (m, 1H), 4.59 – 4.45 (m, 1H), 3.95 (d, *J* = 10.2 Hz, 2H), 3.72 – 3.45 (m, 2H), 3.42 – 3.35 (m, 2H), 3.33-3.29(m, 1H), 2.99-2.87(m, 2H), 2.84 (s, 1.5H), 2.81 (s, 1.5H), 2.68 – 2.50 (m, 6H), 2.47 – 2.25 (m, 4H), 2.12-1.98 (m, 4H), 1.36 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 174.09, 173.89, 173.50, 173.47, 160.21, 151.30, 144.76, 144.69, 126.61, 124.44, 124.43, 124.31, 124.29, 61.08, 61.05, 60.66, 60.61, 51.98, 51.87, 48.53, 46.81, 46.72, 36.29, 36.27, 34.48, 34.26, 34.09, 32.44, 28.37, 28.33, 25.23, 24.44, 20.85, 20.84, 11.15. ESI-MS *m/z*: 499.2 [M + H] $^{+}$. HRMS (ESI) cacld for $C_{26}H_{39}N_6O_2S$ ([M+H]$^{+}$): 499.2857; found: 499.2862.

3,3-difluoro-_N_-((_S_)-3-((1_R_,3_R_,5_S_)-3-(3-isopropyl-5-methyl-4_H_-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(thiophen-2-yl)propyl)clopentane-1-carboxamide (26).

Compound **26** was prepared in a similar manner as described for compound **1**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, *J* = 4.8 Hz, 1H), 7.05 – 6.85 (m, 3H), 5.47 (dd, *J* = 14.1, 6.8 Hz, 1H), 4.36 – 4.24 (m, 1H), 3.42 (d, *J* = 8.7 Hz, 2H), 3.05-2.94

(m, 1H), 2.87-2.73 (m, 1H), 2.60-2.46 (m, 5H), 2.41 – 2.18 (m, 5H), 2.14 – 1.99 (m, 7H), 1.74-1.60 (m, 4H), 1.38 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.37, 159.16, 150.65, 145.72, 145.58, 127.06, 124.32, 124.23, 124.18, 59.05, 58.51, 47.98, 47.19, 42.51, 39.20, 38.94, 38.77, 38.50, 35.69, 35.56, 35.00, 34.96, 34.75, 29.72, 26.91, 26.67, 25.87, 21.67, 13.19. ESI-MS *m/z*: 506.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{26}H_{38}F_2N_5OS$ ($[M+H]^+$): 506.2763; found: 506.2755.

3,3-difluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(thiophen-2-yl)propyl)cyclobutane--1-carboxa mide (27).

Compound **27** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (dd, *J* = 4.6, 1.6 Hz, 1H), 7.00 – 6.93 (m, 2H), 5.43 (dd, *J* = 14.2, 7.1 Hz, 1H), 4.39 – 4.26 (m, 1H), 3.60-3.44 (s, 2H), 3.12 – 2.76 (m, 5H), 2.74 – 2.60 (m, 3H), 2.55 (s, 3H), 2.46-2.27 (m, 2H), 2.25-2.05 (m, 4H), 1.81-1.64 (m, 4H), 1.39 (dd, *J* = 6.8, 1.3 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.61, 159.18, 150.74, 145.22, 127.07, 124.41, 124.35, 121.59, 118.74, 116.07, 58.76, 53.81, 48.13, 47.82, 46.89, 42.07, 38.97, 38.73, 38.50, 38.26, 35.34, 28.13, 27.98, 26.34, 25.85, 21.68, 13.19. ESI-MS *m/z*: 492.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{25}H_{36}F_2N_5OS$ ($[M+H]^+$): 492.2603; found: 492.2589..

4,4-difluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-cyclopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(thiophen-3-yl)propyl)cyclohexane-1-carbox amide (28).

Compound **28** was prepared in a similar manner as described for compound **1**. ^{1}H

NMR (400 MHz, $CDCl_3$) δ 7.32 (dd, *J* = 5.0, 3.0 Hz, 1H), 7.13 (d, *J* = 1.9 Hz, 1H), 7.01 (dd, *J* = 5.0, 1.1 Hz, 1H), 6.47 (s, 1H), 5.26 (dd, *J* = 14.4, 6.9 Hz, 1H), 4.59 – 4.38 (m, 1H), 3.44 (d, *J* = 11.8 Hz, 2H), 2.56 – 2.35 (m, 5H), 2.24 – 2.12 (m, 3H), 2.11 – 2.00 (m, 3H), 1.98 – 1.75 (m, 8H), 1.75 – 1.62 (m, 4H), 1.34 – 1.20 (m, 2H), 1.16 – 0.94 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.33, 155.86, 150.95, 142.89, 126.60, 126.10, 121.07, 58.24, 47.62, 42.98, 34.99, 34.80, 33.08, 32.83, 32.60, 31.60, 26.78, 26.05, 25.96, 22.67, 14.14, 12.57, 6.92, 6.88, 6.61. ESI-MS *m/z*: 518.2 [M + H] $^+$. HRMS (ESI) cacld for $C_{27}H_{38}F_2N_5OS$ ($[M+H]^+$): 518.2762; found: 518.2752.

4,4-difluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-cyclopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(thiophen-2-yl)propyl)cyclohexane-1-carboxamide (29).

Compound **29** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (dd, *J* = 4.9, 1.2 Hz, 1H), 6.96 (dt, *J* = 6.5, 3.3 Hz, 2H), 6.70-6.50 (m, 1H), 5.46 (q, *J* = 6.9 Hz, 1H), 4.56 – 4.42 (m, 1H), 3.45 (d, *J* = 20.1 Hz, 2H), 2.60 – 2.37 (m, 6H), 2.22 – 2.07 (m, 6H), 1.96-1.76 (m, 6H), 1.740-1.64(m, 5H), 1.31 – 1.25 (m, 2H), 1.13-0.99(m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.25, 155.87, 150.96, 145.70, 127.09, 124.98, 124.24, 122.58, 120.19, 58.16, 47.58, 47.47, 42.83, 34.99, 34.78, 33.04, 32.79, 32.55, 31.60, 26.78, 26.01, 25.91, 25.82, 22.67, 14.15, 12.54, 6.96, 6.91, 6.64. ESI-MS *m/z*: 518.2 [M + H] $^+$. HRMS (ESI) cacld for $C_{27}H_{38}F_2N_5OS$ ($[M+H]^+$): 518.2763; found: 518.2758.

4,4-difluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-ethyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(thiophen-3-yl)propyl)cyclohexane-1-carboxamide

(30).

Compound **30** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (dd, *J* = 5.0, 3.0 Hz, 1H), 7.14 (d, *J* = 2.5 Hz, 1H), 7.02 (dd, *J* = 5.0, 1.2 Hz, 1H), 6.65-6.46 (m, 1H), 5.25 (q, *J* = 7.0 Hz, 1H), 4.34 – 4.18 (m, 1H), 3.47 (d, *J* = 8.7 Hz, 2H), 2.76 (q, *J* = 7.5 Hz, 2H), 2.60-2.47 (m, 5H), 2.35 – 2.25 (m, 2H), 2.22 – 2.04 (m, 8H), 1.97-1.76 (m, 5H), 1.75-1.61 (m, 5H), 1.37 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ173.50, 155.61, 150.86, 142.79, 126.62, 126.13, 124.96, 122.62, 121.13, 120.19, 59.27, 58.41, 47.99, 47.53, 42.92, 35.18, 35.03, 33.08, 32.83, 32.59, 29.71, 26.58, 26.04, 25.95, 22.67, 19.62, 14.15, 12.90, 12.15. ESI-MS *m/z*: 506.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{26}H_{38}F_2N_5OS$ ($[M+H]^+$): 506.2763; found: 506.2755.

4,4-difluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-trifluoromethyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(5-methylthiophen-3-yl)propyl)cyclohexane-1-carboxamide (31).

Compound **31** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (400 MHz, $CDCl_3$) ： δ 6.73 (d, *J* = 3.3 Hz, 1H), 6.62-6.59 (m, 1H), 6.16 (d, *J* = 6.6 Hz, 1H), 5.37 (dd, *J* = 14.2, 6.9 Hz, 1H), 4.62-4.48 (m, 1H), 3.40 (d, *J* = 22.7 Hz, 2H), 3.31-3.16 (m, 1H), 2.54 – 2.37 (m, 5H), 2.31 – 2.11 (m, 5H), 2.11 – 1.97 (m, 4H), 1.96-1.90 (m, 2H), 1.89-1.70 (m, 6H), 1.69-1.60(m, 2H), 1.45 (dd, *J* = 6.8, 4.1 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 124.99, 124.17, 59.29, 58.51, 49.43, 48.28, 47.54, 42.86, 36.69, 36.53, 35.39, 33.03, 32.78, 32.54, 26.70, 26.45, 26.38, 25.96, 25.88, 22.37, 22.34, 15.37. ESI-MS *m/z*: 572.2 $[M + H]^+$. HRMS (ESI) cacld for

$C_{28}H_{39}F_5N_5OS$ ($[M+H]^+$): 588.2793; found: 588.2788.

4,4-difluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-isopropyl-5-trifluoromethyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(thiophen-2-yl)propyl)cyclohexane-1-carboxamide (32).

Compound **32** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.20 (m, 1H), 7.01 – 6.93 (m, 2H), 6.29 (d, *J* = 7.5 Hz, 1H), 5.49 (dd, *J* = 14.3, 6.8 Hz, 1H), 4.64 – 4.45 (m, 1H), 3.40 (d, *J* = 23.3 Hz, 2H), 3.25 (dt, *J* = 13.5, 6.7 Hz, 1H), 2.52 – 2.38 (m, 2H), 2.30 – 2.15 (m, 5H), 2.11 – 1.99 (m, 4H), 1.98-1.89 (m, 2H), 1.88 – 1.73 (m, 6H), 1.71-1.61(m, 2H), 1.45 (dd, *J* = 6.7, 4.7 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.10, 163.15, 145.68, 127.09, 124.30, 124.28, 59.28, 58.63, 58.55, 49.43, 48.24, 47.36, 42.82, 38.82, 36.68, 36.50, 35.51, 33.01, 32.77, 32.52, 26.70, 26.40, 25.96, 25.88, 22.37, 22.34. ESI-MS *m/z*: 574.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{27}H_{37}F_5N_5OS$ ($[M+H]^+$): 574.2633; found: 574.2622.

4,4-difluoro-*N*-((*S*)-3-((1*R*,3*R*,5*S*)-3-(3-cyclopropyl-5-trifluoromethyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(thiophen-3-yl)propyl)cyclohexane-1-carboxamide (33).

Compound **33** was prepared in a similar manner as described for compound **1**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (dd, *J* = 5.0, 2.9 Hz, 1H), 7.11 (s, 1H), 7.02 – 6.97 (m, 1H), 6.16 (d, *J* = 7.7 Hz, 1H), 5.30 (dd, *J* = 14.1, 6.8 Hz, 1H), 4.71 – 4.52 (m, 1H), 3.40 (d, *J* = 12.9 Hz, 2H), 2.62 – 2.37 (m, 4H), 2.24-2.11 (m, 3H), 2.10 – 1.64 (m, 15H), 1.34 – 1.14 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.19, 159.34,

144.38, 143.99, 142.97, 126.62, 126.12, 124.95, 122.55, 121.03, 119.98, 58.83, 58.64, 49.60, 48.05, 47.29, 42.98, 35.70, 35.51, 34.52, 33.05, 32.82, 32.59, 26.53, 26.10, 26.00, 25.91, 9.23, 7.68. ESI-MS *m/z*: 572.2 $[M + H]^+$. HRMS (ESI) cacld for $C_{27}H_{35}F_5N_5OS$ ($[M+H]^+$): 572.2477; found: 572.2476.

4,4-difluoro-*N*-(3-((1*R*,3*S*,5*S*)-3-(3-isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl)-1-(thiophen-3-yl)propyl)cyclohexane-1-carboxamide (34)

Compound **34** was prepared in a similar manner as described for compound **1**. ^1H NMR (500 MHz, $CDCl_3$) δ 7.31 (dd, *J* = 5.0, 2.9 Hz, 1H), 7.13 (d, *J* = 2.9 Hz, 1H), 7.01 (dd, *J* = 4.9, 1.4 Hz, 1H), 4.29 (tt, *J* = 12.0, 5.5 Hz, 1H), 3.41 (s, 2H), 2.98 (p, *J* = 6.8 Hz, 1H), 2.51 (s, 5H), 2.37 – 1.97 (m, 10H), 1.97 – 1.55 (m, 11H), 1.38 (dd, *J* = 6.8, 2.1 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.25 , 159.11 , 150.61 , 142.95 , 126.53 , 126.12 , 121.04 , 77.23 , 59.02 , 58.40 , 47.99 , 47.63 , 47.18 , 42.93 , 35.52 , 35.34 , 34.51 , 33.02 , 32.82 , 26.72 , 26.03 , 25.96 , 25.86 , 21.67 , 13.21. ESI-MS *m/z*: 520.3 $[M + H]^+$. HRMS (ESI) cacld for $C_{27}H_{39}F_2N_5OS$ ($[M+H]^+$): 520.2916; found: 520.2917.

***exo*-8-benzyl-8-azabicyclo[3.2.1]octan-3-one (36).**

To a solution of **35** (1.25 g, 10 mmol) in tetrahydrofuran (30 mL) was added sodium carbonate (2.12 g, 20 mmol) and benzyl bromide (1.79 g, 10.5 mmol). The mixture was stirred at room temperature for 2 h. The mixture was extracted with dichloromethane. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in vacuum to give the crude product **36** as a yellow liquid (2.12 g,

97%). ESI-MS *m/z*: 216.1 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, *J* = 6, 2H), 7.30 (t, *J* = 6, 2H), 7.21 (d, *J* = 5.9, 1H), 3.68 (s, 2H), 3.39 (m, 2H), 2.59 (dd, *J* = 3.4, 16.1, 2H), 2.11 (dd, *J* = 1.0, 17.1, 2H), 2.01 (m, 2H), 1.52 (d, *J* = 5.9, 2H).

***exo*-8-benzyl-8-azabicyclo[3.2.1]octan-3-one oxime (37).**

To a solution of **36** (2.16 g, 10 mmol) in acetonitrile (50 mL) was added potassium carbonate (2.76 g, 20 mmol) and hydroxylamine hydrochloride (1.04 g, 15 mmol), The mixture was stirred at room temperature for 2 h. The mixture was extracted with dichloromethane. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in vacuum to give the crude product **37** as a yellow solid (2.31 g, 99%). ESI-MS *m/z*: 231.1 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 8.12 (brs, 1H), 7.31 (m, 2H), 7.28 (m, 2H), 7.17 (m, 1H), 3.55 (s, 2H), 3.26 (m, 2H), 2.90 (d, *J* = 12, 1H), 2.49 (dd, *J* = 3.0, 14.7, 1H), 2.21 (dd, *J* = 3.0, 12, 1H), 2.11 (d, *J* = 11.7, 1H), 1. 91 (m, 2H), 1.48 (m, 2H).

***exo*-8-benzyl-8-azabicyclo[3.2.1]octan-3-amine (38).**

To a solution of **37** (2.31 g, 10 mmol) in *n*-pentanolin (50 mL) was added sodium metal in pieces (2.76 g, 120 mmol) at ambient temperature, and the mixture was heated to reflux. The resultant mixture was heated at reflux for 6 h to ensure complete consumption of sodium. the reaction was allowed to cool to ambient temperature, concentrated hydrochloric acid was added to adjust the aqueous layer to pH 2. The layers were separated, dichloromethane was added to the aqueous layer, which was adjusted to pH 12 by the addition of 2 M aqueous sodium hydroxide. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in

vacuum to give the crude product **38** as a yellow oil (1.95 g, 90%). ESI-MS *m/z*: 217.1 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 (m, 2H), 7.31 (m, 2H), 7.21-7.15 (m, 1H), 3.54 (s, 2H), 3.14 (s, br, 2H), 2.90 (m, 1H), 2.10 (m, br, 2H), 1.71-1.54 (m, br, 2H), 1.48 (d, *J* = 3.0, 2H), 1.38 (m, 2H), 1.30 (m, 2H).

***N*-(*exo*-8--benzyl-8-azabicyclo[3.2.1]octan-3-yl)isobutyramide (39a).**

To a solution of **38** (2.17 g, 10 mmol) in dichloromethane (30 mL) was added sodium carbonate (1.59 g, 15 mmol), and cooled to 0 °C, isobutyryl chloride (1.6 g, 15 mmol) was added over 2 min. The mixture was stirred at room temperature for 2 h. The mixture was extracted with dichloromethane. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in vacuum. The residue was purified by silica gel column (DCM/MeOH, 10/1) to afford **39a** (2.87 g, 99%)。 ESI-MS *m/z*: 287.1 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.30 (m, 5H), 5.05 (m,1H), 4.02 (m, 1H), 3.10 (s, 2H), 2.17 (m, 1H), 2.01 (m, 2H), 1.70 (m, 2H), 1.60 (m, 2H), 1.60 (s, 2H), 1.37 (m, 2H), 1.17 (d, *J* = 3.0, 6H).

***N*-(*exo*-8--benzyl-8-azabicyclo[3.2.1]octan-3-yl)cyclopropanecarboxamide (39b).**

Compound **39b** was prepared in a similar manner as described for compound **39a**. ESI-MS *m/z*: 285.1 $[M + H]^+$.

***N*-(*exo*-8--benzyl-8-azabicyclo[3.2.1]octan-3-yl)propionamide (39c).**

Compound **39c** was prepared in a similar manner as described for compound **39a**. ESI-MS *m/z*: 273.1 $[M + H]^+$.

***exo*-8-benzyl-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octane (40a).**

To a solution of **39a** (1.43 g, 5 mmol) in dichloromethane (20 mL) was added PCl_5 (1.248 g, 5 mmol) over 0.5 h keeping the temperature below 0 °C. The mixture was stirred at room temperature for 2 h. A solution of acetic hydrazide (0.74 g, 10 mmol) in 2-methyl-2-butanol (5 mL) was added over 0.5 h keeping the temperature below 0 °C. The mixture was stirred at room temperature for 16 h. The mixture was concentrated under reduced pressure, distilling and replacing with toluene (20 mL). Acetic acid (6 mg) was added and heated to 80 °C for 2 h. The mixture was cooled to 0 °C and treated with 2 M sodium hydroxide to adjust the aqueous phase to pH 12. The layers were separated, and the aqueous layer was extracted with dichloromethane. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in vacuum. The residue was purified by silica gel column (DCM/MeOH, 10/1) to afford **40a** (1.19 g, 67%). ESI-MS *m/z*: 325.2 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.30-7.25 (m, 5H), 4.35 (m, 1H), 3.61 (s, 2H), 3.39 (s, 2H), 3.17 (m, 1H), 2.61 (s, 3H), 2.30-2.11 (m, 4H), 1.72 (m, 4H), 1.41 (d, *J* = 3.0, 6H).

***exo*-8-benzyl-3-(3-cyclopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octane (40b).**

Compound **40b** was prepared in a similar manner as described for compound **40a**. ESI-MS *m/z*: 323.2 $[M + H]^+$.

***exo*-8-benzyl-3-(3-ethyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octane (40c).**

Compound **40c** was prepared in a similar manner as described for compound **40a**.

ESI-MS *m/z*: 311.2 $[M + H]^+$.

***exo*-8-benzyl-3-(3-isopropyl-5-trifluoromethyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octane (40d)**.

Compound **40d** was prepared in a similar manner as described for compound **39a**. ESI-MS *m/z*: 379.2 $[M + H]^+$.

***exo*-8-benzyl-3-(3-cyclopropyl-5-trifluoromethyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octane (40e)**.

Compound **40e** was prepared in a similar manner as described for compound **40a**. ESI-MS *m/z*: 377.2 $[M + H]^+$.

***exo*-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octane(41a).**

To a solution of **40a** (0.325 g, 1 mmol) in methanol (10 mL) was added 20% palladium hydroxide (32.5 mg) and ammonium formate (0.535 g, 8.5 mmol). The mixture was stirred at 60°C for 2 h. The mixture was cooled to room temperature, filtered and washed with methanol for three times. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in vacuum. The residue was purified by silica gel column (DCM/MeOH, 10/1) to afford **41a** (0.22 g, 92%). ESI-MS *m/z*: 235.2 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 4.33 (m, 1H), 3.64 (s, 2H), 3.31 (s, 2H), 3.20 (m, 1H), 2.60 (s, 3H), 2.30-2.15 (m, 4H), 1.71 (m, 4H), 1.40 (d, J = 3.0, 6H).

***exo*-3-(3-cyclopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octane (41b).**

Compound **41b** was prepared in a similar manner as described for compound **41a**.

ESI-MS *m/z*: 233.2 $[M + H]^+$.

***exo*-3-(3-ethyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octane (41c).**

Compound **41c** was prepared in a similar manner as described for compound **41a**. ESI-MS *m/z*: 221.2 $[M + H]^+$.

***exo*-3-(3-isopropyl-5-trifluoromethyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octane (41d)**.

Compound **41d** was prepared in a similar manner as described for compound **41a**. ESI-MS *m/z*: 289.2 $[M + H]^+$.

***exo*-3-(3-cyclopropyl-5-trifluoromethyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octane (41e)**.

Compound **41e** was prepared in a similar manner as described for compound **41a**. ESI-MS *m/z*: 287.2 $[M + H]^+$.

(*Rs, E*)-2-methyl-N-(thiophen-3-ylmethylene)propane-2-sulfinamide (43a).

To a solution of **42a** (1.12 g, 10 mmol) in tetrahydrofuran (20 mL) was added (*R*)-(+)-2-methyl-2-propanesulfinamide (1.33 g, 11 mmol) and titanium ethoxide (4.56 g, 20 mmol). The mixture was stirred at room temperature for 2 h. The mixture was extracted with dichloromethane. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in vacuum to give the crude product **43a** (1.99 g, 92%) as a light yellow solid. ESI-MS *m/z*: 216.2 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 8.66 (t, *J* = 1.7 Hz, 1H), 7.57 (ddd, *J* = 9.4, 5.2, 4.2 Hz, 1H), 7.52 (dt, *J* = 4.2, 2.1 Hz, 1H), 7.13 (td, *J* = 5.2, 3.5 Hz, 1H), 1.23 (s, 1H).

(*Rs, S*)-tert-butyl (3-oxo-1-(thiophen-3-yl)propyl)carbamate (44a).

A solution of *N*,*N*-diisopropylamine (1.13 mL, 8 mmol) in tetrahydrofuran (10 mL) was cooled to -40 °C, 2.4 M *n*-butyllithium (3.3 mL, 8 mmol) was added slowly. The mixture was stirred at -40 °C for 0.5 h and cooled to -78 °C. Then, methyl acetate (0.58 g, 8 mmol) was added, the mixture was stirred at -78 °C for 0.75 h. 2 M chlorotitanium triisopropoxide (8 mL, 16 mmol) was added, the mixture was stirred at -78 °C for 0.5 h. **43a** (0.86 g, 4 mmol) was added, the mixture was stirred at -78 °C for 3 h and quenched with 10 ml of saturated ammonium chloride solution. The mixture was filtered over celite, and washed with ethyl acetate. The filtrate was concentrated under vacuum. The residue was purified by silica gel column (PE/EA, 2/1) to afford **44a** (0.88 g, 74%). ESI-MS *m/z*: 290.2 $[M + H]^+$.

(*S*)-methyl 3-((tert-butoxycarbonyl)amino)-3-(thiophen-3-yl)propanoate (45a).

To a solution of **44a** (2.89 g, 10 mmol) in methanol (20 mL) was added 4 M hydrochloric acid 1,4-dioxane solution (10 mL). The mixture was stirred at room temperature for 2 h and evaporated in vacuum. Then, triethylamine (2.8 mL, 20 mmol) and di-*tert*-butyl dicarbonate (3.26 g, 15 mmol) were added. The mixture was stirred at room temperature for 3 h and evaporated in vacuum. The mixture was extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in vacuum. The residue was purified by silica gel column (PE/EA, 10/1) to afford **45a** (2.43g, 87%). ESI-MS *m/z*: 286.1 $[M + H]^+$.

(*S*)-*tert*-butyl (3-hydroxy-1-(thiophen-3-yl)propyl)carbamate (46a).

To a solution of **45a** (280 mg, 1 mmol) in tetrahydrofuran (5 mL) was added 1.0 M lithium aluminium hydride (1.1 mL, 1.1 mmol) under 0 °C. The mixture was stirred

at room temperature for 2 h and quenched with 10 ml of 2 M sodium hydroxide solution. The mixture was extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in vacuum to afford crude product **46a** as a colorless liquid (254 mg, 98%). ESI-MS *m/z*: 258.1 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.33 - 7.27 (m, 1H), 7.13 (s, 1H), 7.02 (d, *J* = 4.9 Hz, 1H), 4.99 (s, 2H), 3.68 (dd, *J* = 7.5, 2.5 Hz, 2H), 3.43 (s, 1H), 2.13 (d, *J* = 6.6 Hz, 1H), 1.78 (s, 1H), 1.43 (d, *J* = 7.9 Hz, 11H).

(*S*)-*tert*-butyl (3-oxo-1-(thiophen-3-yl)propyl)carbamate (47a).

To a solution of **46a** (258mg, 1 mmol) in dichloromethane (5 mL) was added Dess-Martin periodinane (466.4 mg, 1.1 mmol) The mixture was stirred at room temperature for 2 h and quenched with 10 ml of saturated sodium bicarbonate solution. The mixture was extracted with dichloromethane. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in vacuum. The residue was purified by silica gel column (PE/EA, 5/1) to afford **47a** (155 mg, 61%). ESI-MS *m/z*: 256.1 $[M + H]^+$.

(*S*)-*tert*-butyl(3-(4-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)piperidin-1-yl)-1-(thiophen-3-yl)propyl)carbamate (48a).

To a solution of **47a** (502 mg, 2 mmol) in dichloromethane (5 mL) was added **41a** (416 mg, 2 mmol) and sodium triacetoxyborohydride (466 mg, 2.2 mmol). The mixture was stirred at room temperature for 12 h and quenched with 10 ml of water. The mixture was extracted with dichloromethane. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in vacuum. The residue was purified

by silica gel column (DCM/MeOH, 20/1) to afford **48a** (668 mg, 70%). ESI-MS *m/z*: 474.3 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.29 - 7.24 (m, 1H), 7.09 (s, 1H), 6.98 (dd, *J* = 5.0, 1.1 Hz, 1H), 6.07 (s, 1H), 4.93 (s, 1H), 4.33 - 4.19 (m, 1H), 3.36 (d, *J* = 33.2 Hz, 2H), 2.96 (dt, *J* = 13.8, 6.8 Hz, 1H), 2.54 (s, 3H), 2.48 - 2.14 (m, 4H), 2.03 (dd, *J* = 16.0, 12.0 Hz, 3H), 1.87 (s, 1H), 1.62 (t, *J* = 9.0 Hz, 4H), 1.47 – 1.30 (m, 16H).

Biological evaluation

Calcium flux assay.

HEK293 cells stably expressing Gα16 and CCR5 were seeded onto 96-well plates and incubated for 24 h. Cells were loaded with 2 μM Fluo-4 AM in Hanks' balanced salt solution (HBSS) at 37˚C for 45 min. After removal of excess dye, 50 μL HBSS containing various concentrations of testing compounds was added. After incubation at room temperature for 10 min, 25 μL HBSS containing RANTES (final concentration 10 nM) was dispensed into the wells using a FlexStation III microplate reader (Molecular Devices), and intracellular calcium change was recorded at an excitation wavelength of 485 nm and an emission wavelength of 525 nm. Data were analyzed with GraphPad Prism software. Nonlinear regression analysis was performed to generate dose-response curves and calculate the IC_{50} values.

Cells and virus.

TZM-bl and HOS-CD4-CCR5 cells were obtained from by NIH AIDS Reagent Program and cultured with DMEM medium containing 10% new born calf serum (NCS, Life technology). PM1 cells were gifted by Prof. Xulin Chen (Wuhan Institute

of Virology, Chinese of Academy of Sciences) and maintained in RPMI 1640 medium with 10% NCS. Peripheral blood mononuclear cells (PBMCs) were separated from healthy donor by Ficoll-Hypaque density gradient centrifugation as manufacturer's instructions description and stimulated by the addition of 5 μg/ml phytohemagglutinin (PHA, Sigma) for 72h and cultured in RPMI-1640 containing 10% FBS and 50 unit/mL IL-2.

CCR5-tropism HIV-1 lab-adapted strains HIV-1_{SF162} HIV-1_{Ba-L} and clinical isolate HIV-1_{KM018} were propagated by co-culture with stimulated PBMCs. Cultured supernatants were collected and filtered with 0.22 μm MILLEX® GP Filter Unit and stored at -70°C with small aliquots. HIV-1 strains HIV-1_{SF162}, was obtained from NIH AIDS Reagent Program, HIV-1_{Ba-L} was kindly donated by Prof. Una O' Doherty (University of Pennsylvania). The clinical isolate strain HIV-1_{KM018} were isolated from local AIDS patients in Yunnan, China before antiviral drug treatment. [25-26]

Cytotoxicity of the CCR5 antagonists.

The cytotoxicity of CCR5 antagonists on TZM-bl cells, PM1 cells, HOS-CD4-CCR5 cells, and PBMCs was determined by MTT assay. Briefly, 2×10^4 per well TZM-bl or PM1 cells (5×10^5 per well for PBMCs,) incubate with or without series diluted compounds in 96-well cell culture plates and incubated for 48 hours (5 days for PM1 and 7 days for PBMCs). For HOS-CD4-CCR5, 1×10^4 cells per well were plated into 96-well cell culture plates and incubated at 37°C, 5% CO_2 overnight. Then the diluted compounds were added and cells were incubated at 37°C, 5% CO_2 for 5 days. MTT was added to each well with final concentration of 0.5 mg/mL and incubated

for 4 hours at 37°C. 100 μL of supernatant was discarded and 100 μL 15%SDS-50%DMF was added and then incubated overnight at 37°C. The optical absorbance was measured by ELx800 reader (Bio-Tek, USA) at 570 nm/630 nm. 50% cytotoxicity concentration (CC_{50}) was calculated.

Antiviral activity assays.

Drug susceptibility assays were performed in 96-well tissue culture plates. 2×10^4 TZM-bl cells per well were infected with HIV-1_{SF162} or HIV-1_{Ba-L} co-incubated with series diluted compounds. 48 hours post infection, cells were lysed with Glo Lysis Buffer (Promega) and luciferase activity of lysate was measured by D-Luciferin potassium (Meilunbio). The relative luciferase activity was measured by using FlexStation III.

1×10^4 per well HOS-CD4-CCR5 cells were seeded in 96-well plates and incubated at 37°C overnight. The cells were treated with compounds and infected with HIV-1_{Ba-L} or HIV-1_{SF162}. 4 hours post infection, free virus was removed and cells were washed once with PBS. Diluted compounds were added to 96-well plates containing infected cells and cultured for 5 days. The supernatant was collected and lysed with 0.5% Triton X100 (Sigma-Aldrich) and the p24 antigen was determined by using an in-house ELISA assay. [27]

2×10^5/mL PM1 cells or 5×10^6/mL stimulated PBMCs were infected with lab-adapted strains or clinical isolates and incubated with diluted compounds for 4 hours at 37°C. Free viru was discarded, cells were washed once and added to each well containing diluted compounds. Cells were incubated for 5 days (PM1) or 7 days (PBMC). The

supernatant was collected and lysed with 0.5% Triton X100 (Sigma-Aldrich) and the p24 antigen was quantified.

Pharmacokinetic profiles in SD rats.

Compounds **1**, **6**, **20**, **21**, and **34** (5% DMSO + 5% Tween-80 in 90% saline) were subjected to PK studies on SD rats. Test compounds were administered *via* the oral route at 20 mg/kg or administered *via* the intravenous route at 10 mg/kg. After oral and intravenous administration, blood samples were collected. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with methanol containing an internal standard. After centrifugation, the supernatant was diluted with methanol and centrifuged again. The compound concentrations in the supernatant were measured by LC/MS/MS.

Metabolites determination in liver cell.

The HRMS instrumentation for sample analysis consisted of anAcquity UPLC system (Waters Corporation, MA, USA) equippedwith a binary solvent manager, a sample manager, a columnmanager, a degasser, and TUV detector, and a Synapt Q-TOFmass spectrometer (Waters) equipped with electrospray ioniza-tion source. Data acquisition was performed using Masslynx V4.1software (Waters), and data analysis was accomplished usingMetaboLynx and MassFragmentTMsoftware (Waters).

hERG testing assay.

Compounds **1**, **6**, **20**, **21**, and **34** stock solution were thawed and 3-fold serial diluted

to a work solution in DMSO, then 500-fold diluted into external solution to get the final solution (20, 6.66, 2.22, 0.74, 0.24 and 0.082 μM). For extracellular application, the compound-containing external solution was delivered to the recording cell using RSC-160 rapid solution changer with a 9-tube head (BioLogic Co.) through gravity-driven. Compound was administered for 60 s after stable hERG potassium current was observed. Each cell was received from 1-6 escalating concentrations. Each concentration was tested on at least 3 cells ($n \geqslant 3$).

Currents were recorded in CHO cells stably expressing hERG potassium channels. During recording, cells were perfused with extracellular solution. Electrodes (a tip resistance of 3-5 MegOhm) were pulled from borosilicate grass pipette (Sutter instrument BF150-86-10) and filled with intracellular solution. Data were obtained using an Axopatch 200B amplifier (Molecular Devices), signals were filtered at 2 kHz and sampled at frequencies of 10 kHz using pClamp 10 software. The cells were held at -80 mV and hERG potassium currents were activated by a 2 s depolarization potential of +20 mV followed by a repolarization potential of -50 mV for 1s then back to the holding potential. Experiments were performed at room temperature.

Data were retrieved and analyzed using pClamp 10, GraphPad Prism 5 and Excel software. The peak amplitude of hERG currents were measured using clampfit and exported to Excel and GraphPad Prism 5 for subsequent analysis. The concentrations of compounds to yield 50 % block of the currents (IC_{50}) were obtained by fitting normalized concentration-inhibition relationships to the equation: $I/Io = 1/\{1+([C]/IC_{50})^n\}$, where Io and I are the current amplitudes measured in control

and in the presence of compounds, [C] is the concentration of compound in the external solution and n is the Hill coefficient. The ratio of inhibition was calculated by using the equation: Inhibition = (1-I/Io) ×100 %, where Io and I are the current amplitude in control and in the presence of compounds, respectively. n is not less than 3 cells for each concentration. Extracellular buffer (mM)：140 NaCl, 5 KCl, 1 $CaCl_2$, 1.25 $MgCl_2$, 10 HEPES and 10 Glucose，pH 7.4 with NaOH. Intracellular buffer (mM)：140 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 EGTA and 10 HEPES，pH 7.2 with KOH

Methods summary for crystallization and structure determination.

The modified CCR5 was expressed in insect cell and membrane was prepared as preciously desctibed.[20] Membrane solubilization was followed by incubation with 200 μM of compounds **21** and **34** at 4 ^{o}C for 1 h. In purification procedure, 100 μM compounds **21** and **34** was added in wash buffer, then the concentration was increased to 300 μM in elution buffer. At last, the protein was treated overnight at 4 ^{o}C with 1 mM compounds **21** and **34** and His-tagged PreScission protease (home-made) to remove the C-terminal His-tag. The His-tag cleaved protein was further purified by Ni-NTA superflow resin (Qiagen) and concentrated to 40-50 mg /ml. Crystals were grown in lipidic cubic phase (LCP) using 9.9 MAG and the precipitant solution consisted of 30-45 % PEG 400, 100 mM HEPES pH 7.5, and 100 mM ammonium acetate. X-ray diffraction data were collected at the SPring-8 beam line 41XU, Hyogo, Japan, and the structure was solved by molecular replacement.

ASSOCIATED CONTENT

Accession Codes

The coordinates for the two crystal structures reported in this paper have been deposited with RCSB, which will be immediately released upon publication. The PDB entry codes for crystal structures reported in this work are as follows: **21**, 6AKX; **34**, 6AKY.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

HPLC analysis data of all target compounds, X-ray Crystallography Data Collection and Refinement Statistics (PDF)

Molecular formula strings (CSV)

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ACKNOWLEDGMENTS

We gratefully acknowledge the funds from National Program on Key Basic Research Project of China (2012CB910704 and 2015CB910304), the National Natural Science Foundation (81321092, 91229205, 81102461, 81102483, 81620108027, 21632008, and 21402226), the China Marine Commonweal Research Project (201005022-5), the Institutes for Drug Discovery and Development, Chinese Academy of Sciences (No. CASIMM0120161006 and CASIMM0320163020), and National S&T Major Projects (2012ZX09301001-007, 2009ZX09501-029, and 2014ZX10005-002).

ABBREVIATIONS

CCR5, CC-chemokine receptor 5; HIV, human immunodeficiency virus; AIDS, acquired immune deficiency syndrome; HAART, highly active antiretroviral therapy; hERG, human *ether-à-go-go* related gene; CYP450, cytochrome P450; PBMC, peripheral blood mononuclear cells; HOS, human osteosarcoma; PM1, T-lymphoid cells; C_{max}, the maximum plasma concentration; AUC, the area under the plasma concentration–time curve; TI, therapeutic index.

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