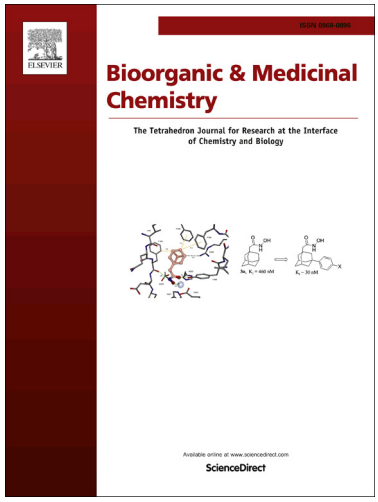

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Design, Synthesis, and Biological Evaluation of Novel 2-methylpiperazine derivatives as Potent CCR5 Antagonists

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Abstract

Three series of novel 2-methylpiperazine derivatives were designed and synthesized using a fragment-assembly strategy. Among them, six compounds (**13**, **16**, **18**, **22**, **33**, and **36**) showed potent activity against CCR5 comparable to that of the positive control, maraviroc, in calcium mobilization assay. Moreover, some compounds were selected and further tested for their antiviral activity in HIV-1 single cycle assay. As a result, four compounds (**13**, **16**, **33**, and **36**) showed antiviral activity at the nanomolar level. Additionally, the potent four compounds showed no cytotoxicity at a concentration of 10 μM.

Keywords: CCR5 antagonist; Anti-HIV-1 agent; 2-Methylpiperazine derivatives

1. Introduction

Acquired immunodeficiency syndrome (AIDS), caused by human immunodeficiency virus (HIV), is one of the fatal diseases threatening human. Although highly active antiretroviral therapy (HAART), a combination of drugs targeting HIV-1 protease and reverse transcriptase, has been successful in reducing HIV-1-associated mortality and morbidity, many patients are still suffered from long term toxicity, incomplete efficacy, and the eventual emergence of resistance. [1] Thereby, it seems urgent to develop novel classes of antiretroviral agents with superior potency and less toxicity.

The chemokine receptor 5 (CCR5), a member of the seven-transmembrane G-protein coupled receptor family, serves a critical role for the entrance of R5-tropic HIV-1 into host cells.[2]As such, the close association between CCR5 and HIV-1 that has been observed provides a foundation for novel methods aimed at controlling HIV-1 infection.[3] This, in turn,has stimulated a great deal of effort in the identification of new CCR5 antagonists. As a result, several novel CCR5 antagonistshave become available. For example, maraviroc (**1**, Fig. 1) was the first marketed small molecular CCR5 antagonist[4] andTAK-220 (**2**, Fig. 1), which was developed by the Takeda Pharmaceutical Co. Ltd., has potent anti-HIV-1 activity at nanomolar concentrations.[5]

In our previous work, we designed a series of novel piperazinederivatives.[6]Among themwas a CCR5 antagonist containing a 4-cyanophenylpiperazin moiety with an IC_{50} value of 6.29 μM(**3,** Fig.1).Furtherresearch resulted in the identification of two additionalpiperazine derivatives with CCR5 antagonist activity, **4** and **5**(see Fig. 2).These two additional compounds were designed using a fragment-assembly strategy(Fig.2) combining the structural features of TAK-220and compound **3**(Part A, **2**; Part B,**3**). Interestingly, evaluation of the CCR5 antagonist activity in a calcium mobilization assay revealed that the 2-methylpiperazin derivative **5**(IC_{50} = 163.8 nM) exhibited better CCR5 inhibitor activity thanderivative **4** (IC_{50} = 239.5 nM), which displayed only moderateactivity.

In order to further explore the structure–activity relationship between 2-methylpiperazin derivatives and potential CCR5 antagonists that may havetherapeutic potential, compound **5** was chosen as our lead compound; we subsequently designed three series of target compounds. First, we replaced the substituent (R_1) at the 4-position of the 2-methylpiperazine ring in compound **5** with a variety of aromatic rings. Then, we focused on modifyingthe terminal group tethered to the amide moiety (R_4). Finally, the 2-methylpiperazine moiety was replaced with aheptatomic ring and bridged ring. Herein, we report the synthesis and biological evaluation of these novel 2-methylpiperazin

derivatives.

2. Results and discussion

2.1. Chemistry

The required *N*-substituted piperazines**7a-k** and **9** were synthesized as depicted in scheme **1**. Specifically, *N*-alkylation of the 2-methylpiperazine **6a** with a substituted benzyl chloride in the presence of potassium carbonate afforded the 1-benzyl-3-methylpiperazine analogues **7a-f**. In contrast, treatment of2-methylpiperazine **6a** with 4-fluorobenzoyl chloride or 4-fluorobenzenesulfonyl chloride resulted in the generation ofthe benzamide**7g** and sulfonamide **7h**, respectively. Moreover, reacting 4-fluorobenzaldehyde with homopiperazine**6b**, (*R*)-2-methylpiperazine **6c**, (*S*)-2-methylpiperazine **6d** or(1*S*,4*S*)-2,5-diazabicyclo[2.2.1]heptanes**8** in the presence of sodium triacetoxyborohydride led to the formation of compounds**7i**, **7j**, **7k**, and **9**, respectively.

The preparation of target compounds **13-22, 26-29** and **32-36**is outlined in schemes**2** and **3**. *N*-alkylation of anilines **10a-c** with 1-bromo-3-chloropropane in the presence of potassium iodide under microwave irradiation provided the corresponding substituted secondary amines **11a-c**. Subsequently, acylation of **11a-c** with 1-acetylpiperidine-4-carbonyl chloride led to formation ofthe amides **12a-c**. Furthermore, we used the*N*-substituted 2-methylpiperazine analogues **7a-h**to react with the amides **12a-c** in the presence of potassium iodide and potassium carbonate in orderto produce the target compounds **13-22**(scheme **2**).

Coupling of 3-chloro-4-methylaniline **10a** with different carboxylic acids gave amides **23a-e**. Afterward, *N*-alkylation of **23a-e** with 2-(2-bromoethyl)-1, 3-dioxolane in the presence of sodium hydride afforded the dioxolane derivatives **24a-e**, which were subsequently treated with hydrochloric acid to produce the aldehydes **25a-e**. Finally, treatment of the aldehydes **25a-d** with 1-(4-fluorobenzyl)-3-methylpiperazine **7d** in the presence of sodium triacetoxyborohydride generated target compounds **26-29**.

The acid chloride **30**[7] was reacted with 3-chloro-*N*-(3-chloropropyl)-4-methylaniline **11a** to provide **31**, then the reaction of compound **31** with 1-(4-fluorobenzyl)-3-methylpiperazine **7d** yielded compound **32**.Compounds **33-36** were prepared from aldehydes**25e** and **9** or **7i-k** with the same synthetic route as compounds **26-29** (scheme **3**).

2.2. Calcium mobilization assay

All compounds were evaluated for antagonistic activity of RANTES induced CCR5 calcium ion mobilization using maraviroc as the positive control.No CCR5 agonist activitywas observed for any of the test compounds.Furthermore, we tested all compounds for purity, ensuring that all compounds were greater than 95% pure. The results are summarized in Table **1**, **2**, and **3**.

As shown in Table **1,** most of the tested compounds displayed potent CCR5antagonism in thisseries. Among them, compounds **13-18**, which contained a benzyl moiety at the 4-position of the 2-methylpiperazine ring, exhibited improved activities compared to the reference structure **5** (IC_{50} = 163.8 nM). In particular, compound **16**(IC_{50} = 6.6 nM) with a 4-fluorobenzyl group had the highest CCR5 binding affinity of all the compounds tested. Compound **13** (IC_{50} = 8.3 nM) with a 4-cyanobenzyl group showed similar potency compared to **16**. However, when a benzyl or 4-methylbenzyl group was placed in the R_1position, the potency was reduced compared to compound **16**. This is demonstrated by the activity of compound **14**(IC_{50} = 15.1 nM) and**15**(IC_{50} = 25.1 nM), respectively, and may indicate that an appropriate orientation of the electron-withdrawing group might be required for high potency. Changing the position of the fluorine atomon the phenyl ring (**17**, *o*-F: IC_{50} = 30.1 nM and**18**, *m*-F: IC_{50} = 11.7 nM) also resulted in a loss of potency compared to **16**. Furthermore, replacement of the 4-fluorobenzyl group in **16** with a 4-fluorobenzoyl group (**21**: IC_{50} = 30.0 nM) led to a 4-fold decrease in potency, while the alteration of the 4-fluorobenzyl group to a 4-fluorobenzenesulfonyl group (**22**: IC_{50} = 10.9 nM) only had a slight loss of potency. This suggests that compounds with a 4-fluorobenzyl group might be more effective to enhance CCR5 activity.

The substituent found at the central phenyl moiety (R_2, R_3)of the target compounds alsoaffected the antagonist activity for CCR5. For example, compound **19,** which lacks asubstituent on the central phenyl ring (IC_{50} = 319.9 nM), showed a 48-fold decrease in potency compared tocompound**16**. Furthermore, compound **20** (IC_{50} = 25.1 nM),which contains only achlorine atom at the 3-position of the central phenyl ring, displayed an approximate 3-fold loss in potency compared to that of compound **16**. These results suggest that a 3-chloro-4-methylphenyl group might be beneficial forretaining high potency for CCR5.

Table 2 summarizes the activity of compounds obtained via alteration of the terminal group tethered to the amide moiety (R_4). Compounds **26-29** and **32** in this series showed lower activity than that of compound **16**, which contains a 1-acetylpiperidine-4-yl moiety at the R_4 position. Compound **26** (IC_{50} = 2158 nM), which contains a methyl group at the R_4 position, displayed a drastic loss in potency

compared to compound**16**. Although the 4-fluorophenyl group found at the R_4 position of compound **27**(IC_{50} = 204.1nM) partly compensated for the loss of CCR5 antagonism observed for compound **26**, it was still considerably less potent than **16**--indicating that the 1-acetylpiperidine moiety might be important for retaining potency. We also examined structural alterations at the piperidine ring to determine the optimum replacement of alternate moieties. Replacing the 1-acetylpiperidine-4-yl moiety on **16** with a 1-acetylpiperidine-3-yl moiety (**28**, IC_{50} = 259.7 nM) or 1-(methylsulfonyl)piperidine moiety (**29**, IC_{50} = 17.1nM) led to a decrease in potency compared to compound **16**. In addition, introduction of a benzoylpiperidine-4-yl moiety to replace the 1-acetylpiperidine-4-yl moiety on **16** resulted in a one-fold reduction in potency (**32**, IC_{50} = 33.2 nM), suggesting that a bulky group at the *para*-position reduces the activity for CCR5 as seen in compound**32**compared to compound**16**. These data provide strong evidence that the 1-acetyl piperidine moiety is vital for maintaining high activity.

The results observed due to an alteration of the 2-methylpiperazine moiety are summarized in Table 3. Compound **33** (IC_{50} = 8.8 nM), containing a (1*S*, 4*S*)-2, 5-diazabicyclo [2.2.1]heptane group showed a slight loss in activity compared to **16**. Additionally, the introduction of a homopiperazine moiety (**34**, IC_{50} = 906.0 nM) to replace the 2-methylpiperazine group of compound**16** resulted in a 137-fold decrease in potency. This indicates that the 2-methylpiperazine moiety might be used as an appropriate basic center. Subsequently, we investigated the chiral center on the 2-methylpiperazine moiety. It has been demonstrated that the ***S***-enantiomer (**36**: IC_{50} = 3.0 nM) exhibits much higher activity than the ***R***-enantiomer (**35**: IC_{50} = 49.9 nM). The difference in activity between the two compounds (**35** and **36**) might stem from the conformational restriction of the methyl group of the 2-methylpiperazine moiety.

2.3. Viral infectivity assays

In an attempt to assess the antagonistic activities of the target compounds against a viral infection, the most potent compounds **13**, **16**, **33**, and **36** were selected for further analysis using the anti-HIV-1 single cycle antiviral assay. As shown in Table **4,** all of the tested compounds showed nanomolarantagonistic activities. The *S*-Enantiomer **36** showed a one-fold increase inantagonisticactivity compared to its raceme **16**, an observation thatis consistent with that of the CCR5 antagonism results.

2.4. Cell Cytotoxicity Assays

In order to evaluate cytotoxicity of the most potent compounds **13**, **16**, **33**, and **36**, we treated

human macrophage cells with 10 μM of each compound and then utilized the CCK-8 assay to measure inhibition rates(data and methodsareincludedin supplementarymaterials).

3. Conclusions

In summary, three series of novel 2-methylpiperazine derivatives were designed and synthesizedby using a fragment-assembly strategy. The CCR5 antagonistic activities of the target compounds were evaluated based on a calcium mobilization assay.Several compounds showed promising activities, with IC_{50} values ranging from 3.0 to 11.7 nM. Among them, six compounds (**13**, **16**, **18**,**22**,**33**,and**36**) showed better activity in comparison with that of the positive control, maraviroc. The most potent compounds, as identified by the calcium mobilization assay, were selected for further antiviral evaluation. As a result, four compounds showedpotent efficacy at the nanomolar level (**13**: IC_{50} = 47.2 nM, **16**: IC_{50} = 140.6 nM,**33**: IC_{50} = 31.4 nM and **36**: IC_{50} = 75.1 nM, respectively). Additionally, the four compounds chosen for additional study (**13**, **16**, **33**, and **36**) showed no cytotoxicityat a concentration of 10 μM by the CCK-8 assay. We believe that the results of this research will provide a foundation that will prove useful in the further design and optimization of novel small molecular CCR5 antagonists.

4. Experimental protocols

4.1. Chemistry

4.1.1 General procedures

Melting points were obtained on a B-540 Buchi melting point apparatus which were uncorrected. ^{1}H- NMR and ^{13}C-NMR spectra were recorded on a Bruker Acance III instrument at 500 MHz (chemical shifts were expressed as δ values relative to TMS as internal standard). Mass spectra (MS) and ESI (positive) were recorded on an Esquire-LC-00075 spectrometer. High-resolution mass spectra (HRMS) were measured by Agilent 6224 LC/MS.

4.1.2 Synthesis of 1, 3-disubstituted piperazine precursors (7a-f): general procedure

A mixture of 2-methylpiperazine (5.00 mmol), substituted benzyl chlorides (5.00 mmol) and K_2CO_3 (7.50 mmol) in DMF (10.00 ml) was stirred at reflux for 12h. The mixture was concentrated *in vacuo*, diluted with water (20.00 ml) and extracted with EtOAc (15.00 ml×3). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by column chromatography on

silica gel (EtOAc/PE 1/2 to 1/0) to afford corresponding compounds **7a-f**.

4.1.2.1 (±)-4-((3-methylpiperazin-1-yl) methyl)benzonitrile (7a). According to the general procedure, the reaction of (±)-2-methylpiperazine with 4-(chloromethyl)benzonitrile produced compound **7a** (0.88g, 82%) as yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.60-7.59 (d, J =8Hz, 2H, Ar-H), 7.45-7.43 (d, J =8Hz, 2H, Ar-H), 3.52 (brs, 2H, CH_2), 2.98-2.85 (m, 3H), 2.70-2.68 (d, J =11Hz, 2H), 2.09-2.03 (m, 1H), 1.89-1.71 (m, 2H), 1.03-1.02 (m, 3H, CH_3). ESI-MS *m/z*: 216 $[M+H]^+$.

4.1.2.2 (±)-1-benzyl-3-methylpiperazine (7b). According to the general procedure, the reaction of (±)-2-methylpiperazine with (chloromethyl)benzene produced compound **7b** (0.80g, 85%) as yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.50-7.32 (m, 5H, Ar-H), 3.51 (s, 2H), 2.97-2.85 (m, 3H), 2.71-2.67 (m, 2H), 2.09-2.05 (m,1H), 1.89-1.71 (m, 2H), 1.03-1.02 (m, 3H). ESI-MS *m/z*: 191 $[M+H]^+$.

4.1.2.3 (±)-3-methyl-1-(4-methylbenzyl)piperazine (7c). According to the general procedure, the reaction of (±)-2-methylpiperazine with 1-(chloromethyl)-4-methylbenzene produced compound **7c** (0.87g, 86%) as yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.19-7.17 (d, *J* =8Hz, 2H, Ar-H), 7.10-7.09 (d, *J* =8Hz, 2H, Ar-H), 3.42 (s, 2H), 2.90-2.81 (m, 3H), 2.75-2.71 (m, 2H), 2.31 (s, 3H, CH_3), 1.96-1.93 (m, 1H), 1.64-1.62 (m, 2H), 0.99-0.95 (m, 3H). ESI-MS *m/z*: 205 $[M+H]^+$.

4.1.2.4 (±)-1-(4-fluorobenzyl)-3-methylpiperazine (7d). According to the general procedure, the reaction of (±)-2-methylpiperazine with 1-(chloromethyl)-4-fluorobenzene produced compound **7d** (0.81 g, 78%) as white oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.27-7.24 (d, J =8Hz, 2H, Ar-H), 6.99-6.96 (d, J =8Hz, 2H, Ar-H), 3.42 (s, 2H, CH_2), 2.94-2.81 (m, 3H), 2.72-2.70 (d, J =11Hz, 2H), 2.00-1.95 (m, 1H), 1.65-1.55 (m, 2H), 0.99-0.98 (m, 3H, CH_3). ESI-MS *m/z*: 209 $[M+H]^+$.

4.1.2.5 (±)-1-(2-fluorobenzyl)-3-methylpiperazine (7e). According to the general procedure, the reaction of (±)-2-methylpiperazine with 1-(chloromethyl)-2-fluorobenzene produced compound **7e**(0.87 g, 84%) as white oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.46-7.44 (m, 1H, Ar-H), 7.34-7.32 (m, 1H, Ar-H), 7.23-7.15 (m, 2H, Ar-H), 3.58 (s, 2H), 2.97-2.86 (m, 3H), 2.79-2.76 (m, 2H), 1.96 (brs, 2H), 1.02-0.99 (m, 3H). ESI-MS *m/z*: 209 $[M+H]^+$.

4.1.2.6 (±)-1-(3-fluorobenzyl)-3-methylpiperazine (7f). According to the general procedure, the reaction of (±)-2-methylpiperazine with 1-(chloromethyl)-2-fluorobenzene produced compound **7f** (0.81 g, 78%) as white oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.46-7.44 (m, 1H, Ar-H), 7.33-7.32 (m, 1H, Ar-H), 7.23-7.14 (m, 2H, Ar-H), 3.58 (s, 2H, CH_2), 2.94-2.85 (m, 3H), 2.79-2.75 (m, 2H), 2.13-2.08 (m,1H), 1.85-1.76 (m, 2H), 1.02-1.00 (m, 3H). ESI-MS *m/z*: 209$[M+H]^+$.

4.1.3 Synthesis of (±)-(4-fluorophenyl)(3-methylpiperazin-1-yl)methanone (7g). To an ice-cooled stirred suspension of (±)-2-methylpiperazine (0.32 g, 3.20 mmol) in DCM (10.00 mL) was added Et_3N (0.41 mL, 3.20 mmol) dropped with 4-fluorobenzoyl chloride (0.47 g, 3.00 mmol) in DCM (5.00 mL), and the mixture was stirred at room temperature for 3 h. Then it was concentrated *in vacuo and water*(20.00 mL) *was added to it.* The residue was extracted by DCM (20 ml×3), the organic layer was combined, then washed with brine (10.00 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc /PE 3/1) to afford the title compound **7g** (0.56 g, 84%) as a light yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.40-7.38 (m, 2H, Ar-H), 7.10-7.06 (m, 2H, Ar-H), 3.60 (s, 2H), 3.00 -2.98 (m, 3H), 2.30-2.28 (m, 1H), 1.89-1.86 (m,1H), 1.49 (brs, 1H), 1.01-0.99 (m, 3H). ESI-MS *m/z*: 223 $[M+H]^+$.

4.1.4 Synthesis of (±)- 1-(4-fluorophenylsulfonyl)-3-methylpiperazine (7h). To an ice-cooled stirred suspension of (±)-2-methylpiperazine (0.32 g, 3.20 mmol) in DCM (10.00 mL) was added Et_3N (0.41 mL, 3.20 mmol) dropped with 4-fluorobenzene-1-sulfonyl chloride (0.58 g, 3.00 mmol) in DCM (5.00 mL), and the mixture was stirred at room temperature for 3 h. Then it was concentrated in vacuo and water (20.00 mL) was added to it. The residue was extracted by DCM (20 ml×3), the organic layer was combined, then washed with brine (10.00 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc /PE 1/1) to afford the title compound **7h.** ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.75-7.74 (m, 2H, Ar-H), 7.20-7.17 (m, 2H, Ar-H), 3.57 (s, 2H), 2.97-2.88 (m, 3H), 2.26-2.25 (m, 1H), 1.88-1.84 (m,1H), 1.46 (brs, 1H), 1.01-0.99 (m, 3H). ESI-MS *m/z*: 259 $[M+H]^+$.

4.1.5 1-(4-fluorobenzyl)-1, 4-diazepane (7i). To a stirred mixture of homopiperazine (0. 60g, 6.00mmol), 4-fluorobenzaldehyde (0. 60g, 5.00mmol) in DCM (15ml) was added AcOH (0.08 ml, 0. 50mmol) and the mixture was stirred at room temperature for 0.5 h. Then Sodium triacetoxyborohydride (1.60g, 7.50mmol) was added in portions. The reaction was stirred at the same temperature for 6h. Finally, the mixture was diluted with saturated aqueous $NaHCO_3$ (15.00ml) followed by water (15.00ml) and extracted with EtOAc (3×15.00ml). The organic layer was dried (MgSO4), filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/PE 5/1) to afford yellow oil (0.90g, yield 77%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.25-7.22 (m, 2H, Ar-H), 6.95-6.92 (m, 2H, Ar-H), 5.15 (brs, 1H), 4.82 (brs, 1H), 3.55-3.53 (m, 2H), 3.48-3.45 (m, 1H), 3.02-3.00 (m,1H), 2.95-2.93 (m,1H), 2.65-2.53 (m, 4H), 1.79-1.76 (m, 2H). ESI-MS

m/z: 209 $[M+H]^+$.

4.1.6 (R)-1-(4-fluorobenzyl)-3-methylpiperazine (7j). According to the procedure of synthesis of 1-(4-fluorobenzyl)-1, 4-diazepane **7i**, the reaction of (*R*)-2-methylpiperazine (0. 60g, 6mmol) with 4-fluorobenzaldehyde (0.60g, 5mmol) produced compound **8j** (1.10g, 86%) as yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.26-7.23 (m, 2H, Ar-H), 6.98-6.94 (m, 2H, Ar-H), 3.41 (s, 2H), 2.94-2.85 (m, 2H), 2.84-2.82 (m, 1H), 2.71-2.69 (m,2H), 2.21 (brs, 1H), 2.01-1.95 (m, 1H), 1.67-1.62 (m, 1H), 1.00-0.98 (d, *J*=6.5Hz, 3H). ESI-MS *m/z*: 209 $[M+H]^+$.

4.1.7 (S)-1-(4-fluorobenzyl)-3-methylpiperazine (7k). According to the procedure of synthesis of 1-(4-fluorobenzyl)-1, 4-diazepane **7i**, the reaction of (*S*)-2-methylpiperazine (0.60g, 6mmol) with 4-fluorobenzaldehyde (0. 60g, 5mmol) produced compound **7k** (1.00g, yield 84%) as yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.26-7.23 (m, 2H, Ar-H), 6.98-6.94 (m, 2H, Ar-H), 3.41 (s, 2H), 2.93-2.87 (m, 2H), 2.85-2.80 (m, 1H), 2.71-2.68 (m, 2H), 1.99-1.94 (m, 1H), 1.76 (brs, 1H), 1.64-1.60 (m, 1H), 0.98-0.97 (d, *J*=6.5Hz, 3H). ESI-MS *m/z*: 209 $[M+H]^+$.

4.1.8 (1S,4S)-2-(4-fluorobenzyl)-2, 5-diazabicyclo [2.2.1] heptanes (9). According to the procedure of synthesis of 1-(4-fluorobenzyl)-1, 4-diazepane **7i**, the reaction of (1*S*,4*S*)-2,5-diazabicyclo [2.2.1]heptanes **8** (0. 6g, 6mmol) with 4-fluorobenzaldehyde (0. 60g, 5.00mmol) produced yellow oil (0.70g, yield 69%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.31-7.28 (m, 2H, Ar-H), 7.00-6.97 (m, 2H, Ar-H), 4.23 (s, 2H, CH_2), 3.69-3.68 (m, 2H), 3.49-3.41 (m, 2H), 3.17-3.13 (m,1H), 2.89-2.82 (m,1H), 1.86-1.83 (m, 1H), 1.72-1.64 (m, 1H). ESI-MS *m/z*: 207 $[M+H]^+$.

*4.1.9 Synthesis of 3,4-disubstituted-N-(3-chloropropyl)aniline (**11a-c**): general grocedure*

A mixture of anilines **10a-c** (9.00 mmol), 1-bromo-3-chloropropane (0.49 g, 3.00 mmol), and KI (0.05 g, 0.30 mmol) in MeCN (5.00 ml) was reacted in microwave reactor at 110 ^{o}C for 15 minutes. The solvent was evaporated *in vacuo*, diluted with water (30 ml), and extracted with EtOAc (30 ml×3). The combined organic layer was dried ($MgSO_4$) and filtered, then the solvent was evaporated *in vacuo.* The residue was purified by column chromatography on silica gel to yield corresponding compounds **11a-c**.

4.1.9.1 3-chloro-N-(3-chloropropyl)-4-methylaniline (11a). Yellow oil (0.49g, 75%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.52 (d, J = 2.3 Hz, 1H), 7.50 (d,J = 8.3 Hz, 1H), 7.38 (m, 1H), 3.73 (m, 2H), 3.53 (2H, m), 2.40 (s, 3H, CH_3), 2.23 (m, 2H,). ESI-MS *m/z*: 219 $[M+H]^+$.

4.1.9.2 N-(3-chloropropyl) aniline (11b). Yellow oil (0.34g, 67%).^{1}H-NMR ($CDCl_3$, 500 MHz)δ:

7.36-7.18 (m, 2H, Ar-H), 7.16-6.99 (m, 3H, Ar-H), 3.68-3.65 (m, 2H, CH_2), 3.60-3.56 (m, 2H, CH_2), 3.03-2.99 (m, 2H, CH_2). ESI-MS *m/z*: 170 $[M+H]^+$.

4.1.9.3 3-chloro-N-(3-chloropropyl)aniline (11c). Yellow oil (0.36g, 60%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.34-7.20 (m, 2H, Ar-H), 7.18-7.00 (m, 2H, Ar-H), 3.67-3.64 (m, 2H, CH_2), 3.57-3.53 (m, 2H, CH_2), 3.05-2.98 (m, 2H, CH_2). ESI-MS *m/z*: 204 $[M+H]^+$.

4.1.10 1-acetyl-N-(3,4-disubstitutedphenyl)-N-(3-chloropropyl)piperidine-4-carboxamide (12a-c): general grocedure.

To an ice-cooled stirred suspension of anilines **11a-c** (1.00 mmol) in DCM (3.00 mL) was added Et_3N (0.40 mL, 3.00 mmol) followed by 1-acetylpiperidine-4-carbonyl chloride (0.23g, 1.20 mmol), and the mixture was stirred at room temperature for 5 h. Then it was poured to a saturated solution of sodium bicarbonate (10.00 mL). The residue was extracted by EtOAc (20.00 ml×3), the organic layer was combined, then washed with water (15.00 ml×3) and brine (10.00 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel to afford the title compound.

4.1.10.1 1-acetyl-N-(3-chloro-4-methylphenyl)-N-(3-chloropropyl)piperidine-4-carboxamide (12a). White solid (88%), mp: 112-114 °C.^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.30 (d, J = 7.7 Hz, 1H), 7.16 (d, J=2.2 Hz, 1H), 6.98 (m, 1H), 4.53 (m, 1H), 3.77 (t, J = 7.1Hz, 2H), 3.65- 3.85 (m, 1H), 3.53 (t, J = 6.6 Hz, 2H), 2.76-2.97 (m, 1H), 2.43 (s, 3H), 2.26-2.49 (m, 2H), 2.03 (s, 3H), 1.52-2.12 (m, 6H). ESI-MS *m/z*: 372 $[M+H]^+$.

4.1.10.2 1-acetyl-N-(3-chloropropyl)-N-phenylpiperidine-4-carboxamide (12b). White solid (80%); mp: 110-112 °C. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.48-7.39 (m, 3H, Ar-H), 7.417-7.15 (m, 2H, Ar-H), 4.50-4.48 (m, 1H), 3.80-3.75 (m, 3H), 3.54-3.52 (t, J = 6.5 Hz, 2H, CH_2), 2.79 (brs, 1H), 2.37-2.29 (m, 2H), 2.04 (s, 3H, CH_3), 2.02-1.98 (m, 2H), 1.75-1.61 (m, 4H). ESI-MS *m/z*: 323 $[M+H]^+$.

4.1.10.3 1-acetyl-N-(3-chlorophenyl)-N-(3-chloropropyl) piperidine-4-carboxamide (12c). Brown solid (88%); mp: 115-117 °C. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.31-6.98 (m, 4H, Ar-H), 4.55-4.50 (m, 1H), 3.83-3.80 (m, 1H), 3.76 (t, J = 7.0 Hz, 2H, CH_2), 3.54 (t, J = 7.0 Hz, 2H, CH_2), 2.94-2.81 (m, 1H), 2.44-2.34 (m, 2H), 2.06 (s, 3H, CH_3), 2.10-2.06 (m, 2H, CH_2), 1.87-1.62 (m, 4H). ESI-MS *m/z*: 357 $[M+H]^+$.

4.1.11 Synthesis of (±)-1-acetyl-N-(3, 4-disubstituted phenyl)-N-(3-substituted-2-substituted piperazin- 1-yl)propyl)piperidine-4-carboxamide (13-22): general procedure

A mixture of 1-acetyl-N-(3, 4-disubstitutedphenyl)-N-(3-chloropropyl)piperidine-4-carboxamide **12a-c** (0.50 mmol), compounds **7a-h** (0.50 mmol), KI (0.05 mmol) and K_2CO_3 (0.75 mmol) in DMF (5.00 ml) was stirred at reflux for 16h. The mixture was concentrated *in vacuo*, diluted with water (20.0 ml) and extracted with EtOAc (15.00 ml×3). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc/PE 1/1 to 1/0) to afford corresponding target compounds **13-22**.

4.1.11.1 (±)-1-acetyl-N-(3-chloro-4-methylphenyl)-N-(3-(4-(4-cyanobenzyl)-2-methylpiperazin-1-yl) propyl)piperidine-4-carboxamide (13). The reaction of **7a** with **12a** produced compound **13** (31.6 mg, 23%) as light yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.58-7.56 (d, *J*= 8.5Hz, 2H, Ar-H), 7.41-7.40 (d, *J*= 8.5Hz, 2H, Ar-H), 7.28-7.26 (m, 1H, Ar-H), 7.15 (m, 1H, Ar-H), 6.95-6.93 (m, 1H, Ar-H), 4.50-4.48 (m, 1H), 3.75-3.72 (m, 1H), 3.63-3.56 (m, 2H), 3.47 (s, 2H), 2.84-2.75 (m, 2H), 2.70-2.53 (m, 4H), 2.40 (s, 3H, CH_3), 2.35-2.30 (m, 2H), 2.22-2.18 (m, 3H), 2.02 (s, 3H, CH_3), 1.92-1.89 (m, 1H), 1.79-1.54 (m, 6H), 1.02-0.94 (m, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 173.81, 168.69, 144.18, 140.89, 136.41, 135.16, 131.99, 131.87, 129.38, 129.26, 128.41, 127.28, 126.27, 118.87, 110.73, 62.21, 60.51, 57.62, 54.83, 53.29, 50.54, 47.96, 45.43, 40.59, 39.26, 29.95, 28.76, 28.26, 24.32, 21.27, 19.69. HRMS (ESI$^+$) m/z calculated for $C_{31}H_{41}ClN_5O_2$ $[M + H]^+$,550.2959; found, 550.2955.

4.1.11.2 (±)-1-acetyl-N-(3-(4-benzyl-2-methylpiperazin-1-yl)propyl)-N-(3-chloro-4-methylphenyl) piperidine -4-carboxamide (14). The reaction of **7b** with **12a** produced compound **14** (63.00 mg, 24%) as light yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.31-7.29 (m, 5H, Ar-H), 7.25-7.23 (m, 1H, Ar-H), 7.16 (brs, 1H, Ar-H), 6.95-6.93 (m, 1H, Ar-H), 4.51-4.49 (m, 1H), 3.76-3.74 (m, 1H), 3.64-3.56 (m, 2H), 3.44 (brs, 2H), 2.84-2.76 (m, 2H), 2.75-2.60 (m, 4H), 2.41 (s, 3H, CH_3), 2.36-2.16 (m, 5H), 2.03 (s, 3H, CH3), 1.92-1.90 (m, 1H), 1.80-1.55 (m, 6H),1.02-0.95 (m, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 173.76, 168.55, 140.73, 135.43, 133.44, 131.92, 130.72, 128.23, 128.23, 127.32, 126.39, 126.24, 124.62, 61.72, 60.52, 56.66, 54.53, 52.85, 50.31, 47.67, 45.47, 40.43, 39.22, 31.39, 28.68, 28.23, 24.21, 21.24, 19.61. HRMS (ESI$^+$) m/z calculated for $C_{30}H_{42}ClN_4O_2$ $[M + H]^+$,525.2996; found, 525.3001.

4.1.11.3 (±)-1-acetyl-N-(3-chloro-4-methylphenyl)-N-(3-(2-methyl-4-(4-methylbenzyl)piperazin -1-yl)propyl)piperidine-4-carboxamide (15). The reaction of **7b** with **12a** produced compound **15** (70.00 mg, 26%) as light yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.26-7.21 (m, 4H, Ar-H),

7.18-7.17 (m, 1H, Ar-H), 7.00-6.96 (m, 2H, Ar-H), 4.51-4.49 (m, 1H), 3.76-3.73 (m, 1H), 3.67-3.58 (m, 2H), 3.47(s, 2H), 2.84-2.72 (m, 2H), 2.71 (s, 3H, CH_3), 2.68-2.52 (m, 4H), 2.40 (s, 3H, CH_3), 2.36-2.17 (m, 5H), 2.03 (s, 3H, CH_3), 1.91-1.90 (m, 1H), 1.81-1.64 (m, 6H), 1.00-0.94 (m, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz) *δ*: 173.76, 168.65, 140.73, 136.38, 135.01, 133.54, 131.73, 130.41, 130.34, 129.13, 128.27, 126.32, 61.83, 60.22, 56.66, 54.85, 52.93, 50.56, 47.81, 45.37, 40.57, 39.21, 31.26, 28.65, 28.23, 24.21, 21.30, 21.17, 19.56. HRMS (ESI^+) m/z calculated for $C_{31}H_{44}ClN_4O_2$ $[M + H]^+$,539.3153; found, 539.3155.

4.1.11.4 (±)-1-acetyl-N-(3-chloro-4-methylphenyl)-N-(3-(4-(4-fluorobenzyl)-2-methylpiperazin-1-yl) propyl)piperidine-4-carboxamide (16). The reaction of **7d** with **12a** produced compound **16** (75.80 mg, 28%) as light yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.27-7.22 (m, 3H, Ar-H), 7.17-7.16 (m, 1H, Ar-H), 6.98-6.93(m, 3H, Ar-H), 4.50-4.47 (m, 1H), 3.76-3.72 (m, 1H), 3.65-3.62 (m, 2H), 3.43 (s, 2H), 2.91-2.57 (m, 6H), 2.40 (s, 3H, CH_3), 2.38-2.36 (m, 2H), 2.32-2.29 (m, 3H), 2.02 (s, 3H, CH_3), 1.93-1.90 (m, 1H), 1.77-1.55 (m, 6H), 1.01-0.95 (m, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz) *δ*: 173.77, 168.65, 162.50, 160.06, 140.81, 136.39, 135.11, 133.53, 131.76, 130.44, 130.37, 129.33, 126.20, 114.92, 114.71, 61.85, 60.25, 56.60, 54.51, 52.89, 50.49, 47.80, 45.37, 40.54, 39.18, 31.35, 28.61, 28.14, 24.10, 21.10, 19.65. HRMS (ESI^+) m/z calculated for $C_{30}H_{41}ClFN_4O_2$ $[M + H]^+$,543.2903; found, 543.2898.

4.1.11.5 (±)-1-acetyl-N-(3-chloro-4-methylphenyl)-N-(3-(4-(2-fluorobenzyl)-2-methylpiperazin-1-yl) propyl)piperidine-4-carboxamide (17). The reaction of **7e** with **12a** produced compound **17** (71.10 mg, 26%) as yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) *δ*: 7.44-7.42 (m, 1H, Ar-H), 7.31-7.25 (m, 2H, Ar-H), 7.20-7.18 (m, 1H, Ar-H), 7.15 (m, 2H, Ar-H), 6.94-6.92 (m, 1H, Ar-H), 4.49-4.46 (m, 1H), 3.74-3.71 (m, 1H), 3.64-3.60 (m, 2H), 3.53 (s, 2H), 2.91-2.81(m, 2H), 2.78-2.73 (m, 2H), 2.67-2.66 (m, 2H), 2.38 (s, 3H, CH_3), 2.34-2.19 (m, 5H), 2.01 (s, 3H, CH_3), 1.98-1.92 (m, 1H), 1.78-1.74 (m, 1H), 1.65-1.54 (m, 5H), 1.00-0.94 (m, 3H, CH_3). HRMS (ESI^+) m/z calculated for $C_{30}H_{41}ClFN_4O_2$ $[M + H]^+$,543.2903; found, 543.2909.

4.1.11.6 (±)-1-acetyl-N-(3-chloro-4-methylphenyl)-N-(3-(4-(3-fluorobenzyl)-2-methylpiperazin-1-yl) propyl)piperidine-4-carboxamide (18). The reaction of **7f** with **12a** produced compound **18** (57.00 mg, 21%) as yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) *δ*: 7.28-7.23 (m, 3H, Ar-H), 7.16 (brs, 1H, Ar-H), 7.07-7.06 (m, 2H, Ar-H), 6.94-6.92 (m, 1H, Ar-H), 4.52-4.49 (m, 1H), 3.76-3.73 (m, 1H), 3.66-3.58 (m, 2H), 3.43 (s, 2H), 2.89-2.85 (m, 3H), 2.68-2.58 (m, 3H), 2.41 (s, 3H, CH_3), 2.36-2.31 (m,

2H), 2.26-2.18 (m, 3H), 2.03 (s, 3H, CH_3), 1.90 (brs, 1H), 1.77-1.55 (m, 6H), 1.02-0.95(m, 3H, CH_3). HRMS (ESI^+) m/z calculated for $C_{30}H_{41}ClFN_4O_2$ $[M + H]^+$,543.2903; found, 543.2907.

4.1.11.7 (±)-1-acetyl-N-(3-(4-(4-fluorobenzyl)-2-methylpiperazin-1-yl)propyl)-N-phenylpiperidine-4 -carboxamide (19). The reaction of **7d** with **12b** produced compound **19** (71.60 mg, 29%) as yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.42-7.25 (m, 7H, Ar-H), 7.18-7.17 (m, 2H, Ar-H), 4.52-4.49 (m, 1H), 3.75-3.73 (m, 1H), 3.64-3.59 (m, 2H), 3.44 (s, 2H), 2.91-2.78 (m, 2H), 2.73-2.59 (m, 4H), 2.41-2.15 (m, 5H), 2.03 (s, 3H, CH_3), 1.92-1.91 (m, 1H), 1.83-1.56 (m, 6H), 1.03-0.97 (m, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 173.71, 168.63, 162.47,160.03, 140.72, 135.13, 133.52, 131.74, 130.44, 129.21, 126.29, 114.81, 114.59, 61.85, 60.23, 56.62, 54.80, 52.89, 50.21, 47.82, 45.32, 40.57, 39.28, 31.31, 28.62, 28.17, 24.30, 21.22. HRMS (ESI^+) m/z calculated for $C_{29}H_{40}ClFN_4O_2$ $[M + H]^+$,495.3135; found, 495.3138.

4.1.11.8 (±)-1-acetyl-N-(3-chlorophenyl)-N-(3-(4-(4-fluorobenzyl)-2-methylpiperazin-1-yl)propyl) piperidine-4-carboxamide (20). The reaction of **7d** with **12c** produced compound **20** (66.10 mg, 25%) as yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.39-7.37 (m, 2H, Ar-H), 7.24-7.23 (m, 1H, Ar-H), 7.18 (s, 1H, Ar-H), 7.05 (brs, 2H, Ar-H), 7.00-6.95 (m, 2H, Ar-H), 4.52-4.49 (m, 1H), 3.77-3.74 (m, 1H), 3.70-3.62 (m, 2H), 3.40 (brs, 2H), 2.98-2.57 (m, 6H), 2.35-2.30 (m, 2H), 2.26-2.16 (m, 3H), 2.03 (s, 3H, CH_3), 1.90-1.56 (m, 7H), 1.03-0.95 (m, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 173.72, 168.63, 162.47, 160.03, 140.71, 136.31, 135.13, 133.57, 131.34, 130.62, 130.43, 129.13, 126.31, 114.82, 114.60, 61.83, 60.32, 56.61, 54.48, 52.91, 50.31, 47.83, 45.39, 40.52, 39.28, 31.35, 28.51, 28.14, 24.29, 21.17. HRMS (ESI^+) m/z calculated for $C_{29}H_{39}ClFN_4O_2$ $[M + H]^+$,529.2745; found, 529.2751.

4.1.11.9 (±)-1-acetyl-N-(3-chloro-4-methylphenyl)-N-(3-(4-(4-fluorobenzoyl)-2-methylpiperazin-1-yl) propyl) piperidine-4-carboxamide (21). The reaction of **7g** with **12a** produced compound **21** (0.064 g, 23%) as yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.46-7.28 (m, 4H, Ar-H), 7.18-7.17 (m, 1H, Ar-H), 7.11-6.98 (m, 2H, Ar-H), 4.52-4.49 (m, 1H), 3.76-3.73 (m, 1H), 3.68-3.62 (m, 2H), 3.19-3.12 (m, 2H), 2.86-2.80 (m, 2H), 2.63-2.59 (m, 2H), 2.41 (s, 3H, CH_3), 2.37-2.06 (m, 5H), 2.03 (s, 3H, CH_3), 1.96-1.50 (m, 7H), 1.02-0.94 (m, 3H, CH_3). HRMS (ESI^+) m/z calculated for $C_{30}H_{39}ClFN_4O_3$ $[M + H]^+$,557.2694; found, 557.2698.

4.1.11.10 (±)-1-acetyl-N-(3-chloro-4-methylphenyl)-N-(3-(4-(4-fluorophenylsulfonyl)-2-methylpiperazin-1-yl)propyl)piperidine-4-carboxamide (22). The reaction of **7h** with **12a**

produced compound **22** (0.077 g, 26%) as yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.54-7.46 (m, 4H, Ar-H), 7.20-7.18 (m, 1H, Ar-H), 7.15-6.99 (m, 2H, Ar-H), 4.51-4.49 (m, 1H), 3.76-3.74 (m, 1H), 3.70-3.69 (m, 2H), 3.20-3.12 (m, 2H), 2.88-2.58 (m, 4H), 2.40 (s, 3H, CH_3), 2.39-2.05 (m, 5H), 2.03 (s, 3H, CH_3), 2.00-1.93 (m, 1H), 1.85-1.59 (m, 6H), 1.02-0.95 (m, 3H, CH_3). HRMS (ESI^+) m/z calculated for $C_{29}H_{39}ClFN_4O_2$ $[M + H]^+$,529.2745; found, 529.2751.

4.1.12 Synthesis of (±)-N-(3-chloro-4-methylphenyl)-N-(3-(4-(4-fluorobenzyl)-2-methylpiperazin-1-yl) propyl)acetamide (26)

4.1.12.1 N-(3-chloro-4-methylphenyl)acetamide (23a). To a solution of 3-chloro-4-methylaniline **10a** (0.85g, 6.00 mmol) in DCM (15.00 mL), EDC·HCl (1.72g, 9.00 mmol) and acetic acid (0.34ml, 6.00 mmol) were added successively at room temperature, and the resulting reaction mixture was stirred for 12h at the same temperature. Then the reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/PE 1/4) to furnish the title compound **23a** (0.84g, yield 76%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.92 (s, 1H, NH), 7.57 (brs, 1H, Ar-H), 7.26-7.23 (m, 1H, Ar-H), 7.11-7.09 (m, 1H, Ar-H), 2.29 (s, 3H, CH_3), 2.14 (s, 3H, CH_3). ESI-MS *m/z*: 184 $[M+H]^+$.

4.1.12.2 N-(2-(1,3-dioxolan-2-yl)ethyl)-N-(3-chloro-4-methylphenyl)acetamide (24a). To an ice-cooled stirred solution of *N*-(3-chloro-4-methylphenyl)acetamide 23**a** (0.65 g, 4.00mmol) in DMF (8.00ml) was added NaH (60% in oil, 160mg, 4.00mmol). After 1 h the mixture was treated with 2-(2-bromoethyl)-1, 3-dioxolane (0.47ml, 4mmol) and stirred at 80 °C for 12 h. The mixture was concentrated in vacuo, diluted with water (20ml), and extracted with DCM (3×20ml). The organic layer was dried (MgSO4), filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/PE 1/1) to give the product (0.81 g, yield 71%) as oil.^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.25-7.23 (d, *J*=8Hz, 1H, Ar-H), 7.18 (brs, 1H, Ar-H), 6.98-6.96 (dd, *J*=2Hz, 8Hz, 1H, Ar-H), 4.88-4.86 (m, 1H), 3.91-3.88 (m, 2H), 3.80-3.77(m, 4H), 2.37 (s, 3H, CH_3), 1.88-1.84 (m, 2H), 1.81 (s, 3H, CH_3). ESI-MS *m/z*: 284 $[M+H]^+$.

4.1.12.3 N-(3-chloro-4-methylphenyl)-N-(3-oxopropyl)acetamide (25a). *N*-(2-(1,3-dioxolan-2-yl)ethyl)-*N*-(3-chloro-4-methylphenyl)acetamide 24**a** (0.79 g, 2.80mmol) was dissolved in1N HCl (10.00ml), and the mixture was stirred at room temperature for 18 h. The mixture was extracted with DCM (3×15.00ml), and the organic layer was dried (MgSO4), filtered, and concentrated in vacuo to give **25a** (0.65g, yield 97%) as an oil.^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 9.74(s,

1H), 7.26(s, 1H), 7.17(brs, 1H, Ar-H), 6.97-6.95(m, 1H, Ar-H), 4.00-3.98 (m, 2H), 2.70-2.66 (m, 2H), 2.39 (s, 3H, CH_3), 1.83 (s, 3H, CH_3). ESI-MS *m/z*: 240 $[M+H]^+$.

4.1.12.4 (±)-N-(3-chloro-4-methylphenyl)-N-(3-(4-(4-fluorobenzyl)-2-methylpiperazin-1-yl) propyl)acetamide (26). To a stirred mixture of *N*-(3-chloro-4-methylphenyl)-*N*-(3-oxopropyl)acetamide 25**a** (0.12g, 0.5mmol), (±)-1-(4-fluorobenzyl)-3-methylpiperazine **7d** (0.10g, 0.5mmol) in DCM (8ml) was added AcOH (0.008 ml, 0.05mmol) and the mixture was stirred at room temperature for 0.5 h. Then Sodium triacetoxyborohydride (0.16g, 0.75mmol) was added in portions. The reaction was stirred at the same temperature for 6h. Finally, the mixture was diluted with saturated aqueous $NaHCO_3$ (10ml) followed by water (10ml) and extracted with EtOAc (3×10ml). The organic layer was dried (MgSO4), filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/PE 3/1) to afford yellow oil (0.071g, yield 33%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.25-7.22(m, 3H, Ar-H), 7.15(brs, 1H, Ar-H), 6.96-6.94(m, 3H, Ar-H), 3.71-3.58(m, 2H), 3.40(brs, 2H), 2.79-2.57 (m, 4H), 2.37 (s, 3H, CH_3), 2.31-2.21 (m, 4H), 1.95-1.89 (m, 1H), 1.81 (s, 3H, CH_3), 1.69-1.66 (m, 2H), 0.98-0.96(m, 3H, CH_3). HRMS (ESI^+) m/z calculated for $C_{24}H_{32}ClFN_3O$ $[M + H]^+$,432.2218; found, 432.2223.

4.1.13 Synthesis of (±)-N-(3-chloro-4-methylphenyl)-4-fluoro-N-(3-(4-(4-fluorobenzyl)-2-methylpiperazin -1-yl) propyl)benzamide (27)

4.1.13.1 N-(3-chloro-4-methylphenyl)-4-fluorobenzamide (23b). According to the method of *synthe*sis of *N*-(3-chloro-4-methylphenyl)acetamide 23**a**, the reaction of **10a** (0.85g, 6.00 mmol) with 4-fluorobenzoic acid (0.84g, 6.00 mmol) produced compound **23b** as a white oil (1.38g, yield 87%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 8.05(s, 1H), 7.85-7.82(m, 2H), 7.67(brs, 1H, Ar-H), 7.37-7.35(m, 1H, Ar-H), 7.15-7.14(m, 1H, Ar-H), 7.11-7.07(m, 2H, Ar-H), 2.33 (s, 3H, CH_3). ESI-MS *m/z*: 264 $[M+H]^+$.

4.1.13.2 N-(2-(1, 3-dioxolan-2-yl) ethyl)-N-(3-chloro-4-methylphenyl)-4-fluorobenzamide (24b). According to the method of synthesis of *N*-(2-(1, 3-dioxolan-2-yl) ethyl)-*N*-(3-chloro-4-methylphenyl) acetamide **24a**, the reaction of **23b** (1.05g, 4.00mmol) with 2-(2-bromoethyl)-1,3-dioxolane (0.47ml, 4mmol) produced compound **24b** as a white oil (1.38g, yield 77%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.30-7.27(m, 2H), 7.09 (brs, 1H, Ar-H), 7.05-7.03(m, 1H, Ar-H), 6.87-6.84(m, 2H, Ar-H), 6.78-6.76(m, 1H, Ar-H), 4.97-4.95(m, 1H), 4.02-3.99(m, 2H), 3.95-3.92(m, 2H), 3.84-3.82(m, 2H), 2.28 (s, 3H, CH_3), 2.02-1.98(m, 2H). ESI-MS *m/z*: 365 $[M+H]^+$.

4.1.13.3 N-(3-chloro-4-methylphenyl)-4-fluoro-N-(3-oxopropyl)benzamide (25b). According to the method of synthesis of *N*-(3-chloro-4-methylphenyl)-*N*-(3-oxopropyl)acetamide 25**a**, the reaction of **24b**(1.02g, 2.80mmol) with 1N HCl produced compound **25b** as a white oil (0.86g, yield 96%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 9.79(s, 1H), 7.30-7.27(m, 2H), 7.09 (brs, 1H, Ar-H), 7.05-7.03(m, 1H, Ar-H), 6.87-6.83(m, 2H, Ar-H), 6.78-6.74(m, 1H, Ar-H), 4.01-3.98(m, 2H), 3.93-3.92(m, 2H), 2.28 (s, 3H, CH_3). ESI-MS *m/z*: 320 $[M+H]^+$.

4.1.13.4 (±)-N-(3-chloro-4-methylphenyl)-4-fluoro-N-(3-(4-(4-fluorobenzyl)-2-methylpiperazin-1-yl) propyl)benzamide (27). According to the method of synthesis of *(±)-N-(3-chloro-4-methylphenyl)-N-(3-(4-(4-fluorobenzyl)- 2-methylpiperazin-1-yl) propyl)acetamide* **26**, the reaction of **25** (0.16g, 0.50mmol)with (±)-1-(4-fluorobenzyl)-3-methylpiperazine **7d** (0.10g, 0.50mmol) produced compound **27** (0.11g, yield 45%)as a yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.29-7.27(m, 2H), 7.26-7.23(m, 2H),7.08 (brs, 1H, Ar-H), 7.29-7.23 (m, 4H, Ar-H), 7.08-6.95 (m, 4H, Ar-H), 6.88-6.84 (m, 2H, Ar-H), 6.74-6.72 (m, 1H, Ar-H), 3.93-3.83(m, 2H), 3.41 (s, 2H), 2.80-2.41(m, 5H), 2.28 (s, 3H, CH_3), 2.25-1.70 (m, 6H), 1.02-0.97 (m, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 169.14, 164.47, 163.17, 161.98, 160.74, 142.12, 134.75, 134.66, 133.77, 133.74, 131.91, 131.88, 131.18, 130.94, 130.85, 130.57, 130.49, 127.68, 126.10, 115.05, 114.83, 62.03, 60.38, 54.90, 53.15, 50.91, 50.58, 49.01, 29.66, 24.10, 19.53. HRMS (ESI^+) m/z calculated for $C_{29}H_{33}ClF2N_3O$ $[M + H]^+$,512.2280; found, 512.2285.

4.1.14 Synthesis of (±)-1-acetyl-N-(3-chloro-4-methylphenyl)-N-(3-(4-(4-fluorobenzyl)-2-methylpiperazin-1-yl) propyl) piperidine-3-carboxamide (28)

4.1.14.1 1-acetyl-N-(3-chloro-4-methylphenyl) piperidine-3-carboxamide (23c). According to the method of *synthe*sis of N-(3-chloro-4-methylphenyl)acetamide 23**a**, the reaction of **10a** (0.85g, 6.00 mmol) with 1-acetylpiperidine-3-carboxylic acid (1.03g, 6.00 mmol) produced compound **23c** (1.49g, yield 84%) as a yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.69 (brs, 1H, Ar-H), 7.33-7.29 (m, 1H, Ar-H), 7.09-7.06 (m, 1H, Ar-H), 4.44-4.41 (m, 1H), 4.15-4.11 (m, 2H), 3.58-3.55 (m, 1H), 3.47-3.24 (m,2H), 2.50-2.45 (m, 1H), 2.26 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 1.94-1.68 (m, 2H), 1.49-1.32 (m, 1H). ESI-MS *m/z*: 295.7 $[M+H]^+$.

4.1.14.2 N-(2-(1, 3-dioxolan-2-yl)ethyl)-1-acetyl-N-(3-chloro-4-methylphenyl)piperidine-3-carboxamide (24c). According to the method of synthesis of *N*-(2-(1, 3-dioxolan-2-yl)ethyl)-*N*-(3-chloro-4- methylphenyl)acetamide 24**a**, the reaction of **23c** (1.18g,

4.00mmol) with 2-(2-bromoethyl)-1,3- dioxolane (0.47ml, 4mmol) produced compound **24c**(1.25g, yield 79%) as a yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.74 (brs, 1H, Ar-H), 7.33-7.30 (m, 1H, Ar-H), 7.13-7.11 (m, 1H, Ar-H), 4.90-4.88 (m, 2H), 4.55-4.46 (m, 2H), 3.69-3.63 (m, 2H), 3.48-3.46 (m,1H), 2.56 (brs, 1H), 2.26-2.22 (m,2H), 2.12 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 1.78-1.74 (m, 4H), 1.73-1.56 (m, 4H). ESI-MS *m/z*: 396 $[M+H]^+$.

4.1.14.3 1-acetyl-N-(3-chloro-4-methylphenyl)-N-(3-oxopropyl) piperidine-3-carboxamide (25c). According to the method of synthesis of *N*-(3-chloro-4-methylphenyl)-*N*-(3-oxopropyl)acetamide **25a**, the reaction of **24c** (1.11g, 2.80mmol) with 1N HCl produced compound **25c** (0.92g, yield 94%) as a yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 9.10-9.09(m, 1H), 7.69(brs, 1H, Ar-H), 7.31-7.29(m, 1H, Ar-H), 7.08-7.06 (m, 1H, Ar-H), 4.44-4.41 (m, 1H), 4.15-4.01 (m, 2H), 3.58-3.55 (m, 1H), 3.47-3.24 (m,2H), 2.49-2.45 (m, 1H), 2.26 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.05-1.68 (m, 5H), 1.54-1.44 (m, 1H). ESI-MS *m/z*: 351 $[M+H]^+$.

4.1.14.4 (±)-1-acetyl-N-(3-chloro-4-methylphenyl)-N-(3-(4-(4-fluorobenzyl)-2-methylpiperazin--1-yl) propyl) piperidine-3-carboxamide (28). According to the method of synthesis of (±)-*N*-(3-chloro-4-methylphenyl)-*N*-(3-(4-(4-fluorobenzyl) -2-methylpiperazin-1-yl) propyl)acetamide **26**, the reaction of **25c**(0.18g, 0.50mmol) with (±)-1-(4-fluorobenzyl)- 3-methylpiperazine **7d** (0.10g, 0.50mmol) produced compound **28**(0.11g, yield 39%) as a yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.73 (brs, 1H, Ar-H), 7.33-7.31 (m, 1H, Ar-H), 7.27-7.24 (m, 2H, Ar-H), 7.11-7.09 (m, 1H, Ar-H), 6.99-6.96 (m, 2H, Ar-H), 4.60-4.45 (m, 1H), 3.97-3.95 (m, 1H), 3.72-3.65 (m, 1H), 3.54-3.50 (m, 1H), 3.42 (brs, 2H, CH_2), 2.95-2.82 (m, 4H), 2.72-2.70 (m, 2H), 2.54-2.40 (m, 2H), 2.29 (s, 3H, CH_3), 2.23-2.16 (m, 1H), 2.11 (s, 3H, CH_3), 2.07-1.86 (m, 5H), 1.82-1.50 (m, 4H), 1.00-0.94 (m, 3H, CH_3). HRMS (ESI^+) m/z calculated for $C_{30}H_{41}ClFN_4O_2$ $[M + H]^+$,543.2902; found, 543.2908.

4.1.15 Synthesis of (±)-N-(3-chloro-4-methylphenyl)-N-(3-(4-(4-fluorobenzyl)-2-methylpiperazin-1-yl) propyl)-1-(methylsulfonyl)piperidine-4-carboxamide (29)

4.1.15.1 1-methylsulfonyl-N-(3-chloro-4-methylphenyl) piperidine-4-carboxamide (23d). According to the method of *synthe*sis of *N*-(3-chloro-4-methylphenyl)acetamide **23a** , the reaction of **10a** (0.85g, 6.00 mmol) with 1-(methylsulfonyl)piperidine-4-carboxylic acid (1.24g, 6.00 mmol) produced compound **23d** (1.63g, yield 82%) as a yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.73(s, 1H), 7.61(brs, 1H, Ar-H), 7.30-7.28 (m, 1H, Ar-H), 7.14-7.13 (m, 1H, Ar-H), 4.61-4.58 (d, *J* =13Hz, 1H), 3.91-3.88 (d, *J* =13Hz, 1H), 3.14-3.09 (m, 1H), 2.71-2.68 (m, 2H), 2.51-2.46 (m, 1H), 2.31 (s,

3H, CH_3), 2.10 (s, 3H, CH_3), 1.93 (brs, 2H), 1.83-1.69 (m, 2H). ESI-MS *m/z*: 332 $[M+H]^+$.

4.1.15.2 N-(2-(1,3-dioxolan-2-yl) ethyl)-N-(3-chloro-4-methylphenyl)-1-(methylsulfonyl) piperidine -4-carboxamide (24d). According to the method of synthesis of *N*-(2-(1, 3-dioxolan-2-yl)ethyl)-*N*-(3-chloro- 4-methylphenyl) acetamide **24a**, the reaction of **23d** (1.32g, 4.00mmol) with 2-(2-bromoethyl)-1,3- dioxolane produced (0.47ml, 4mmol) compound **24d** (1.29g, yield 72%) as a yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.28-7.27 (d, J=8Hz, 1H, Ar-H), 7.20 (brs, 1H, Ar-H), 7.00-6.98 (m, 1H, Ar-H), 4.90-4.88 (m, 1H), 3.93-3.90 (m, 2H), 3.82-3.76 (m, 4H), 3.71-3.68 (m, 2H), 2.72 (s, 3H, CH_3), 2.60-2.54 (m,2H), 2.40 (s, 3H, CH_3), 2.28-2.22 (m, 1H), 1.88-1.84 (m, 4H), 1.69-1.66 (m, 2H). ESI-MS *m/z*: 432 $[M+H]^+$.

4.1.15.3 N-(3-chloro-4-methylphenyl)-1-(methylsulfonyl)-N-(3-oxopropyl)piperidine-4-carboxamide (25d). According to the method of synthesis of *N*-(3-chloro-4-methylphenyl)-*N*-(3-oxopropyl)acetamide **25a**, the reaction of **24d** (1.20g, 2.80mmol) with 1N HCl produced compound **25d** (1.03g, yield 92%) as a yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 9.73 (s, 1H), 7.31-7.29 (d, J=8Hz, 1H, Ar-H), 7.18 (brs, 1H, Ar-H), 6.99 -6.96 (m, 1H, Ar-H), 3.98-3.95 (m, 1H), 3.71-3.67 (m, 2H), 2.72 (s, 3H, CH_3), 2.68-2.65 (m, 2H), 2.58-2.53 (m, 2H), 2.41 (s, 3H, CH_3), 2.28-2.22 (m, 1H), 1.88-1.80 (m, 4H), 1.68-1.64 (m, 2H). ESI-MS *m/z*: 387 $[M+H]^+$.

4.1.15.4 (±)-N-(3-chloro-4-methylphenyl)-N-(3-(4-(4-fluorobenzyl)-2-methylpiperazin-1-yl) propyl)- -1-(methylsulfonyl)piperidine-4-carboxamide (29). According to the method of synthesis of (±)-*N*-(3-chloro-4-methylphenyl)-*N*-(3-(4-(4- fluorobenzyl)- 2-methylpiperazin-1-yl) propyl)acetamide **26**, the reaction of *N*-(3-chloro-4- methylphenyl)-1-(methylsulfonyl)-*N*-(3-oxopropyl)piperidine-4-carboxamide **25d** (0.19g, 0.50mmol) with (±)-1-(4-fluorobenzyl)- 3-methylpiperazine **7d** (0.10g, 0.5mmol) produced compound **29** (0.12g, yield 43%) as yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.28-7.22 (m, 3H, Ar-H), 7.16-7.15 (m, 1H, Ar-H), 6.99-6.93 (m, 3H, Ar-H), 3.71-3.68 (m, 2H), 3.64-3.59 (m, 2H), 3.39 (m, 2H), 2.77-2.75 (m, 1H), 2.71 (s, 3H, CH_3), 2.68-2.63 (m, 2H), 2.58-2.52 (m, 3H), 2.40 (s, 3H, CH_3), 2.37 (brs, 1H), 2.27-2.15 (m, 4H), 1.90-1.81 (m, 3H), 1.67-1.64 (m, 4H), 1.02-0.95(m, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 173.86, 162.65,160.22, 140.73, 136.41, 135.07, 133.49, 131.71, 130.45, 130.39, 128.32, 126.21, 114.97, 114.76, 61.71, 60.53, 56.80, 54.89, 52.92, 50.23, 47.61, 45.69, 40.58, 39.18, 31.38, 28.65, 28.28, 26.21, 21.16, 19.77. HRMS (ESI^+) m/z calculated for $C_{29}H_{41}ClFN_4O_3S$ $[M + H]^+$,579.2572; found, 579.2579.

4.1.16 Synthesis of (±)-1-benzoyl-N-(3-chloro-4-methylphenyl)-N-(3-(4-(4-fluorobenzyl)-

2-methylpiperazin- -1-yl)propyl)piperidine-4-carboxamide (32)

4.1.16.1 1-benzoyl-N-(3-chloro-4-methylphenyl)-N-(3-chloropropyl) piperidine-4-carboxamide (31). To an ice-cooled stirred suspension of 3-chloro-N-(3-chloropropyl)-4-methylaniline **10a** (0.22g, 1.00 mmol) in DCM (3.00 mL) was added Et_3N (0.40 mL, 3.00 mmol) followed by 1-benzoylpiperidine-4-carbonyl chloride **30** (0.30 g, 1.20 mmol), and the mixture was stirred at room temperature for 5 h. Then it was poured to a saturated solution of sodium bicarbonate (10.00 mL). The residue was extracted by EtOAc (20 ml×3), the organic layer was combined, then washed with water (15 ml×3) and brine (10.00 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel to afford yellow oil (0.32g, yield 75%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.38-7.35 (m, 5H, Ar-H), 7.31-7.29 (m, 1H, Ar-H), 7.17 (brs, 1H, Ar-H), 6.98-6.96 (m, 1H, Ar-H), 4.66-4.56 (m, 1H), 3.78-3.75 (m, 2H), 3.54-3.52 (m, 2H), 3.29-3.26 (m, 2H), 2.84-2.53 (m, 2H), 2.42 (s, 3H, CH_3), 2.01-1.99 (m, 2H), 1.79-1.52 (m, 4H). ESI-MS *m/z*: 434 $[M+H]^+$.

4.1.16.2 (±)-1-benzoyl-N-(3-chloro-4-methylphenyl)-N-(3-(4-(4-fluorobenzyl)-2-methylpiperazin--1-yl)propyl)piperidine-4-carboxamide (32). A mixture of 1-benzoyl-*N*-(3-chloro-4-methylphenyl)-*N*- (3-chloropropyl)piperidine-4- carboxamide **31** (0.22g, 0.50 mmol), (±)-1-(4-fluorobenzyl)-3- methylpiperazine **7d** (0.10g, 0.50 mmol), KI (0.008, 0.05 mmol) and K_2CO_3 (0.10g, 0.75 mmol) in DMF (5.00 ml) was stirred at reflux for 16h. The mixture was concentrated *in vacuo*, diluted with water (20.00 ml) and extracted with EtOAc (15 ml×3). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc/PE 1/2) to afford yellow oil (0.08g, yield 29%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.37-7.27 (m, 5H, Ar-H), 7.24-7.14 (m, 2H, Ar-H), 6.99-6.93 (m,3H, Ar-H), 6.60 (brs,1H, Ar-H), 6.44-6.42 (m,1H, Ar-H), 4.62-4.60 (m, 1H), 3.76-3.73 (m, 1H), 3.66-3.63 (m, 2H), 3.42 (s, 2H), 2.82-2.76 (m, 2H), 2.69-2.57 (m, 4H), 2.40 (s, 3H, CH_3), 2.34-2.18 (m, 5H), 1.91-1.90 (m, 1H), 1.80-1.53 (m, 6H), 1.01-0.95 (m, 3H, CH_3). HRMS (ESI^+) m/z calculated for $C_{35}H_{43}ClFN_4O_2$ $[M + H]^+$,605.3058; found, 605.3060.

4.1.17 Synthesis of 1-acetyl-N-(3-chloro-4-methylphenyl)-N-(3-((1S,4S)-5-(4-fluorobenzyl)-2,5-diazabicyclo [2.2.1]heptan-2-yl)propyl)piperidine-4-carboxamide (33). To a mixture of 1-acetyl-*N*-(3-chloro-4-methylphenyl)-*N*-(3-oxopropyl) piperidine-4-carboxamide **25e** (0.18g, 0.5mmol) and *(1S,4S)-2-(4-fluorobenzyl)-2, 5-diazabicyclo [2.2.1] heptanes* **9** (0.10g, 0.50mmol) in DCM (8.00ml) was added AcOH (0.008 ml, 0.05mmol) and the mixture was stirred at room temperature for

0.5 h. Then Sodium triacetoxyborohydride (0.16g, 0.75mmol) was added in portions. The reaction was stirred at the same temperature for 6h. Finally, the mixture was diluted with saturated aqueous $NaHCO_3$ (10.00ml) followed by water (10ml) and extracted with EtOAc (3×10.00ml). The organic layer was dried (MgSO4), filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/PE 1/1) to afford yellow oil (0.11g, yield 41%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.29-7.27 (m, 3H, Ar-H), 7.18-7.17 (m, 1H, Ar-H), 6.98-6.95 (m, 3H, Ar-H), 4.52-4.49 (m, 1H), 3.76-3.73 (m, 1H), 3.71-3.58(m, 4H), 3.27-3.22 (m, 2H), 2.86-2.80 (m, 1H), 2.71-2.58 (m, 5H), 2.45-2.44 (m, 1H), 2.41 (s, 3H, CH_3), 2.38-2.31 (m, 2H), 2.03 (s, 3H, CH_3), 1.77-1.56 (m, 8H). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 173.82, 168.72, 162.94, 160.51, 141.02, 136.36, 135.53, 135.13, 131.85, 129.82, 129.75, 128.46, 126.31, 115.00, 114.79, 61.96, 61.24, 57.44, 56.43, 55.71, 51.46, 47.93, 45.46, 40.63, 39.30, 33.59, 28.77, 28.26, 27.46, 21.31, 19.72. ESI-MS *m/z*: HRMS (ESI$^+$) m/z calculated for $C_{30}H_{39}ClFN_4O_2$ $[M + H]^+$,541.2745; found, 541.2749.

4.1.18 Synthesis of 1-acetyl-N-(3-chloro-4-methylphenyl)-N-(3-(4-(4-fluorobenzyl)-1,4-diazepan-1-yl) propyl)piperidine-4-carboxamide (34). According to the method of synthesis of 1-acetyl-*N*-(3-chloro-4-methylphenyl)-*N*-(3-((1S,4S)-5- (4- fluorobenzyl)-2,5- diazabicyclo [2.2.1]heptan-2-yl)propyl)piperidine-4-carboxamide (**33**), the reaction of 1-acetyl-*N*-(3-chloro-4-methylphenyl)-*N*-(3-oxopropyl) piperidine-4-carboxamide **25e** (0.18g, 0.50mmol) with 1-(4-fluorobenzyl)-1,4-diazepane **7i** (0.10g, 0.50mmol) produced compound **34** (0.12g, 46%) as yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.30-7.26 (m, 3H, Ar-H), 7.18 (brs, 1H, Ar-H), 6.99-6.97(m, 3H, Ar-H), 4.53-4.50 (m, 1H), 4.08 (brs, 2H), 3.76-3.74 (m, 3H), 3.55-3.50 (m, 4H), 3.41(brs, 2H), 2.86-2.80 (m, 1H), 2.69-2.55 (m, 4H), 2.41 (s, 3H, CH_3), 2.36-2.30 (m, 2H), 2.04 (s, 3H, CH_3), 1.85-1.57 (m, 8H). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 174.01, 168.75, 162.85, 160.42, 140.94, 136.53, 135.30, 131.97, 130.22, 129.01, 128.43, 127.90, 126.38, 115.15, 114.94, 62.48, 61.52, 57.70, 55.62, 54.52, 53.39, 50.71, 46.62, 45.49, 40.65, 39.34, 28.83, 28.28, 27.51, 21.34, 19.76. HRMS (ESI$^+$) m/z calculated for $C_{30}H_{41}ClFN_4O_2$ $[M + H]^+$,543.2904; found, 543.2903.

4.1.19 Synthesis of (R)-1-acetyl-N-(3-chloro-4-methylphenyl)-N-(3-(4-(4-fluorobenzyl)-2-methylpiperazin-1-yl)propyl)piperidine-4-carboxamide (35). According to the method of synthesis of 1-acetyl-*N*-(3-chloro-4-methylphenyl)-*N*-(3-((1S,4S)-5- (4- fluorobenzyl)-2,5-diazabicyclo [2.2.1]heptan-2-yl)propyl)piperidine-4-carboxamide (**33**), the reaction of 1-acetyl-*N*-(3-chloro- 4-methylphenyl)-*N*-(3-oxopropyl) piperidine-4-carboxamide **25e** (0.18g,

0.50mmol) with (*R*)-1-(4-fluorobenzyl)-3-methylpiperazine **7j** (0.11g, 0.50mmol) produced compound **35** as yellow oil (0.068g, yield 26%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.28-7.27(m, 2H, Ar-H), 7.17 (m, 1H, Ar-H), 7.00-6.97 (m, 2H, Ar-H), 6.60 (brs, 1H, Ar-H), 6.42-6.40 (m, 1H, Ar-H), 4.45-4.43 (m, 1H), 3.80-3.77 (m, 1H), 3.71-3.60 (m, 2H), 3.44(s, 2H), 2.96-2.81 (m, 6H), 2.41 (s, 3H, CH_3), 2.37-2.29 (m, 2H), 2.24-2.21 (m, 3H), 2.09 (s, 3H, CH_3), 1.91-1.0 (m, 1H), 1.70-1.59 (m, 6H), 1.01-1.00 (d, J =6.5Hz, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 173.71, 168.63, 162.47, 160.04, 140.75, 136.34, 135.08, 133.53, 131.81, 130.45, 130.37, 128.30, 126.20, 114.91, 114.70, 61.88, 60.23, 56.52, 53.41, 52.93, 50.16, 47.29, 45.58, 40.52, 39.13, 31.41, 28.63, 28.16, 24.24, 21.20, 19.19. HRMS (ESI^+) m/z calculated for $C_{30}H_{41}ClFN_4O_2$ $[M + H]^+$,543.2902; found, 543.2906.

4.1.20 Synthesis of (S)-1-acetyl-N-(3-chloro-4-methylphenyl)-N-(3-(4-(4-fluorobenzyl)-2-methylpiperazin1-yl)propyl)piperidine-4-carboxamide (36). According to the method of synthesis of 1-acetyl-*N*-(3-chloro-4-methylphenyl)-*N*-(3-((*1S,4S*)-5- (4- fluorobenzyl)-2,5-diazabicyclo [2.2.1]heptan-2-yl)propyl)piperidine-4-carboxamide (**33**), the reaction of 1-acetyl-*N*-(3-chloro-4-methylphenyl)-*N*-(3-oxopropyl) piperidine-4-carboxamide **25e** (0.18g, 0.50mmol) with (*S*)-1-(4-fluorobenzyl)-3-methylpiperazine **7k** (0.11g, 0.50mmol) produced compound **36** as yellow oil (0.08g, yield 31%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.28-7.22 (m, 3H, Ar-H), 7.16 (s, 1H, Ar-H), 7.02-6.94(m, 3H, Ar-H), 4.51-4.48 (m, 1H), 3.76-3.73 (m, 1H), 3.64-3.62(m, 2H), 3.40 (s, 2H), 2.86-2.57 (m, 6H), 2.40 (s, 3H, CH_3), 2.36-2.31 (m, 2H), 2.27-2.17 (m, 2H), 2.03 (s, 3H, CH_3), 1.90-1.87 (m, 1H), 1.77-1.58 (m, 6H), 0.97-0.95(d, J =6.0Hz, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 173.71, 168.63, 162.49, 160.06, 140.76, 136.33, 135.08, 133.53, 131.76, 130.45, 130.37, 128.33, 126.19, 114.91, 114.70, 61.89, 60.24, 56.60, 54.81, 52.98, 50.51, 47.85, 45.34, 40.53, 39.17, 31.32, 28.65, 28.16, 24.14, 21.19, 19.61. HRMS (ESI^+) m/z calculated for $C_{30}H_{41}ClFN_4O_2$ $[M + H]^+$,543.2902; found, 543.2908.

4.2. Biological assay methods

4.2.1. Calcium mobilization assay

CHO cells stably expressing CCR5 and $G\alpha_{16}$ were loaded with 2.00 μmol/L Fluo-4 AM in Hanks balanced salt solution (HBSS, containing KCl 5.40 mmol/L, Na_2HPO_4 0.30 mmol/L, KH_2PO_4 0.4 mmol/L, $NaHCO_3$ 4.20 mmol/L, $CaCl_2$ 1.30 mmol/L, $MgCl_2$ 0.50 mmol/L, Mg_2SO_4 0.60 mmol/L, NaCl 137.00 mmol/L, BSA 5.00 g/L, 2.5 mmol/L probenacid , Glucose 5.60 mmol/L, Sulfinpyrazone

250 μmol/L, pH 7.4) at 37 °C for 45 minutes. After the cells being rinsed with the reaction buffer, 50 μL HBSS containing known antagonists (positive control), compounds of interest or DMSO (negative control, final concentration 1%) were added. After incubation at room temperature for 10 minutes, 25 μL RANTES (final concentration 3nmol/L) was dispensed into the well using a FlexStation II microplate reader (Molecular Devices, Sunnyvale, CA, USA) and intracellular calcium change was recorded with an excitation wavelength of 485 nm and emission wavelength of 525 nm. The half maximal inhibitory concentrations (IC_{50}) of compounds were determined with GraphPad Prism software by constructing their dose-response curves. All experiments were repeated a total of three times. [8]

4.2.2. Viral infectivity assays

Plasmids: HIV-1 proviral indicator construct pNL-Luc-E- contains a full-length HIV-1 genome, in which env was replaced by firefly Luciferase coding sequence. pENV-Ad8 expresses R5-tropic envelope (AD8).

Viral infectivity assays: Single-cycle HIV-1 replication assays were performed as described previously. In brief, 4×10^5 293T cells were co-transfected with 0.4 μg of pNL-Luc-E- and 0.4 μg of pENV-R5. After 48h, the supernatant containing pseudovirion was harvested by filtration through a 0.45 μm filter and the amount of viral capsid protein was measured by p24 antigen capture ELISA (Biomerieux). The resultant supernatant (10 μL) was used to infect SupT1 cells (1×10^5) in 96-well plates in the presence of testing compound at the concentration indicated. The SupT1 cells were lysed 48h post-infection and firefly luciferase activities were determined using a firefly Luciferase Assay System (Promega). Values were normalized to the control group treated with DMSO and represented relative infectivity of each sample testing. [8]

Acknowledgements

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spectrometry for structure elucidation.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at

Rererences and notes

1. Yeni, P. G; Hammer, S. M.; Carpenter, C. C. J.; Cooper, D. A.; Fischl, M. A.; Gatell, J. M.; Gazzard, B. G; Hirsch, M. S.; Jacobsen, D. M.; Katzenstein, D. A.; Montaner, J. S. G; Richman, D. D.; Saag, M. S.; Schechter, M.; Schooley, R. T.; Thompson, M. A.; Vella, S.; Volberding, P. A. *JAMA* **2002**, *288*, 222.

2. Lederman, M.M.; Penn-Nicholson, A.; Cho M.; Mosier, D. *JAMA* **2006**, *296*, 815.

3. Berger, E. A.; Murphy, P. M.; Farber, J. M. *Annu. Rev. Immunol.* **1999**, *17*, 657.

4. Liu, T.; Weng, Z.; Dong, X.; Hu, Y. *Mini-Rev. Med. Chem.* **2010**, *10*, 1277.

5. Takashima, K.; Miyake, H.; Kanzaki, N.; Tagawa, Y.; Wang, X.; Sugihara, Y.; Iizawa, Y.; Baba, M. *Antimicrob. Agents Chemother.* **2005**, *49*, 3474.

6. Liu, T.; Weng, Z.; Dong, X.; Chen, L.; Ma, L.; Cen, S.; Zhou, N.; Hu, Y. *PLoS ONE* **2013**, *8*, e53636.

7. Rossi, C.; Porcelloni, M.; D'Andrea, P.; Fincham. C. I.; Ettorre, A.; Mauro, S.; Squarcia, A.; Bigioni, M.; Parlani, M.; Nardelli, F.; Binaschi, M.; Maggi, C. A.; Fattori, A D. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2305 – 2308.

8. Hu, S., Gu, Q.; Wang, Z.; Weng, Z.; Cai, Y.; Dong, X.; Hu, Y.; Liu, T.; Xie, X. *Eur J. Med. Chem.* **2014**, *71*, 259-266.

Fig. 1. Structures of Maraviroc, TAK-220, and piperazine derivative **3**.

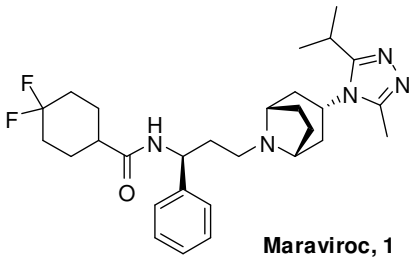
Fig. 2. Design of target compounds.

Scheme 1. Synthesis of *N*-substituted piperazines. Reagents and conditions: (a) substituted benzyl chloride, K_2CO_3, DMF, reflux, 12h; (b) 4-fluorobenzaldehyde, $NaBH(OAc)_3$, DCM, rt, 6h; (c) 4-fluorobenzoyl chloride *or* 4-fluorobenzeneulfonyl chloride , Et_3N, DCM, rt, 4h.

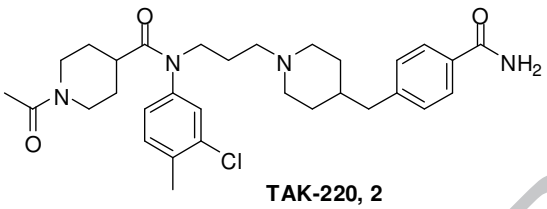
Scheme 2. Synthesis of target compounds **13-22**. Reagents and conditions: (a) 1-bromo-3-chloropropane, K_2CO_3, KI, CH_3CN, 110°C, 0.5h; (b) 1-acetylpiperidine-4-carbonyl chloride, pyridine, DCM, rt, 4h; c) K_2CO_3, KI, DMF, reflux, 12h.

Scheme 3. Synthesis of target compounds **26-29, 32-36.** Reagents and conditions: (a) EDC, R_4COOH, DCM, rt, 12h; (b)2-(2-bromoethyl)-1,3-dioxolane, NaH, DMF, 80°C, 12h; (c) 1N HCl, 18h; (d) $NaBH(OAc)_3$, rt, 6h, (e) Et_3N, DCM, rt, 4h; (f) K_2CO_3, KI, DMF, reflux, 12h.

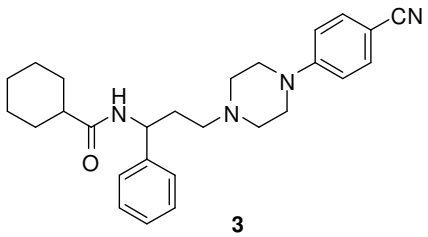
Fig. 1.



Maraviroc, 1

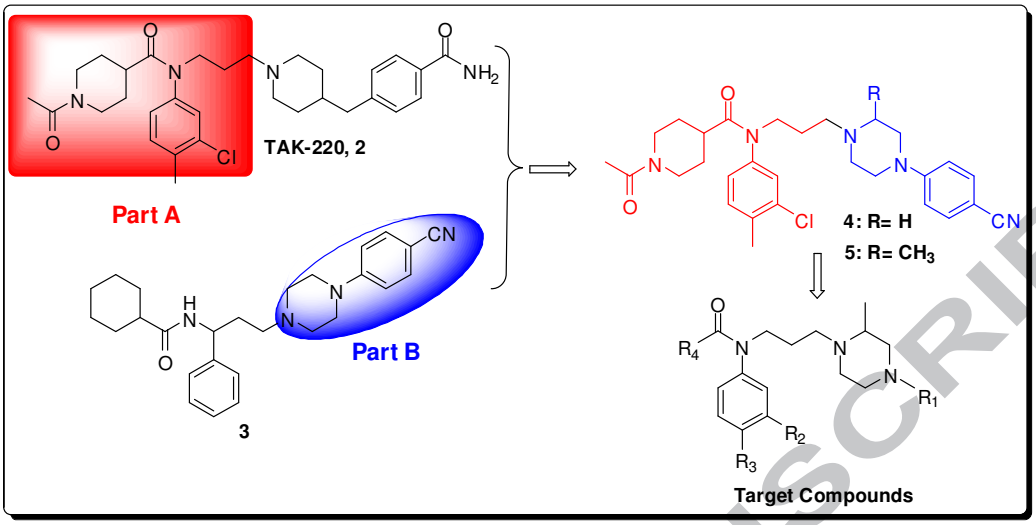


TAK-220, 2

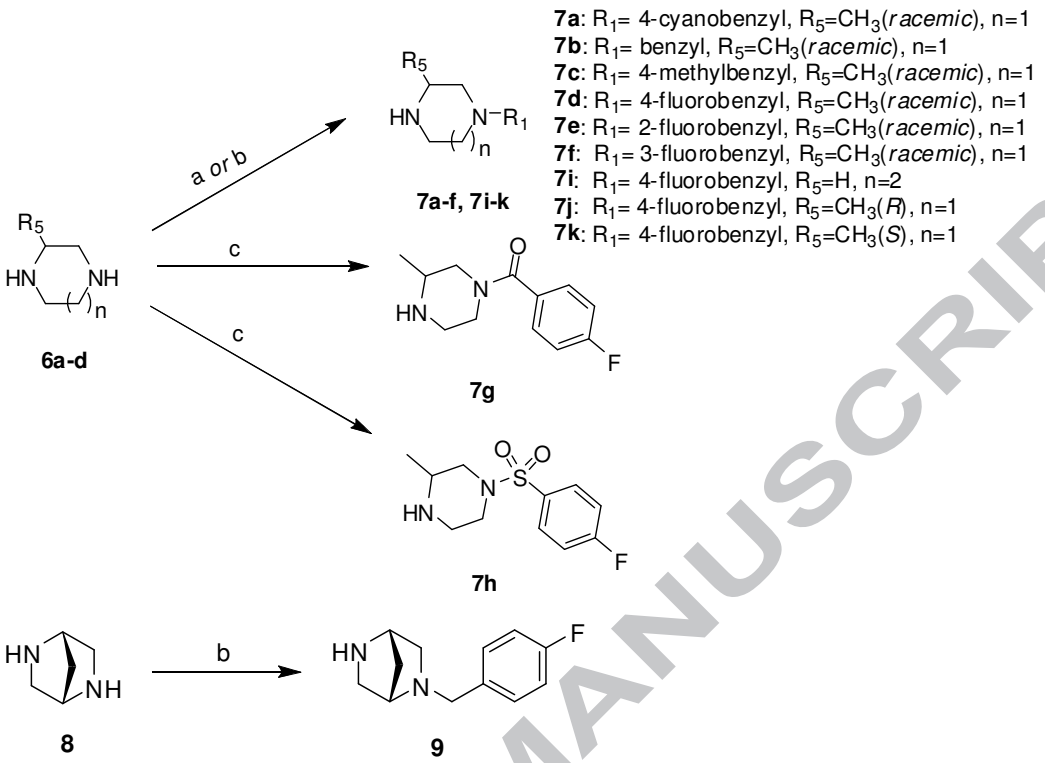


3

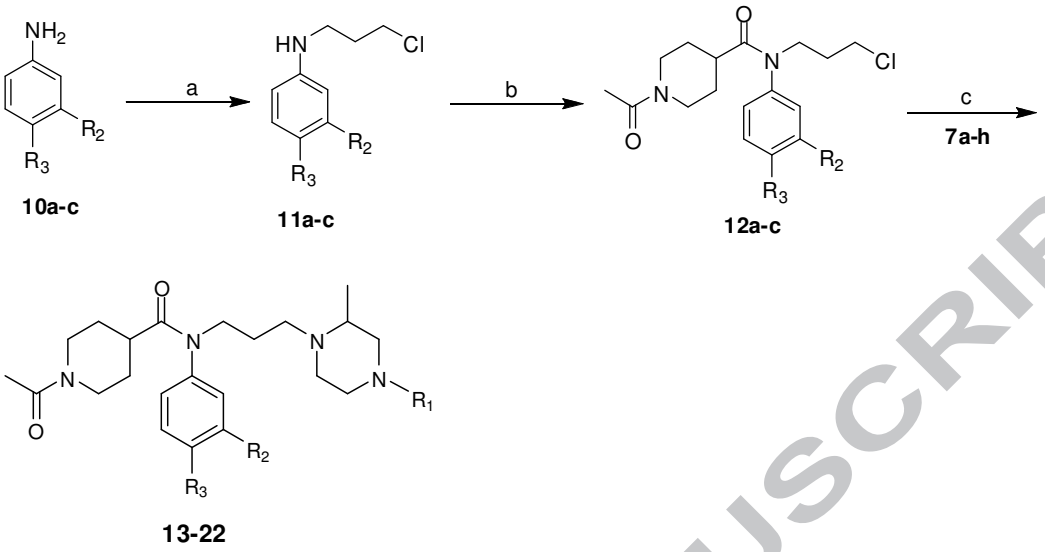
Fig. 2.



Scheme 1.



Scheme 2.



Scheme 3.

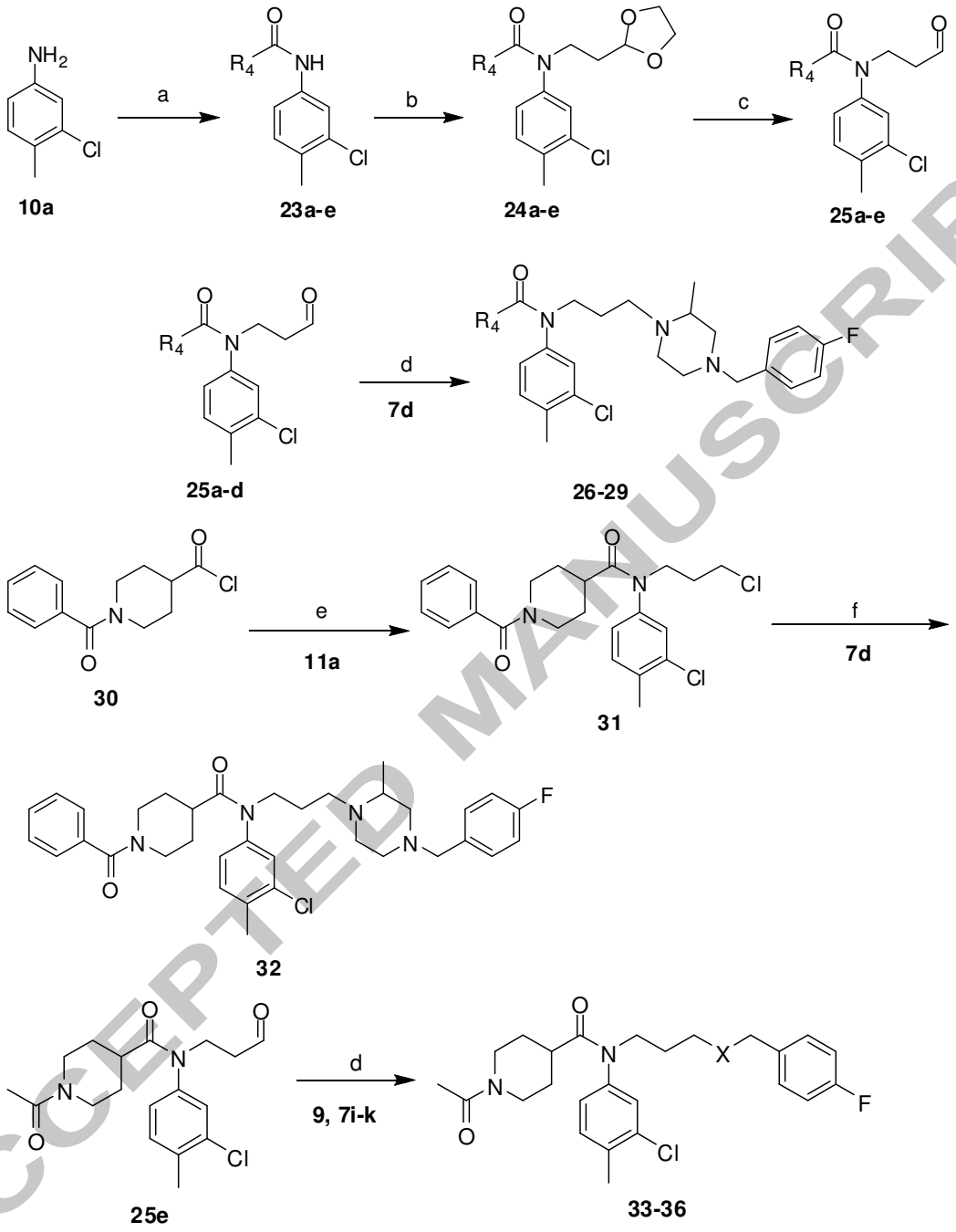


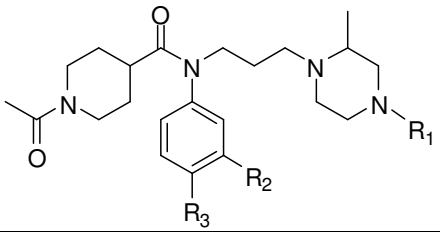
Table 1 Synthesized derivatives **13-22** and their respective CCR5 calcium mobilization antagonism.

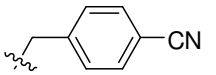
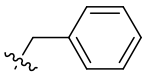
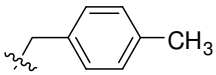
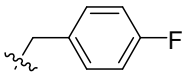
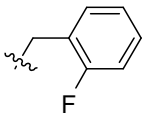
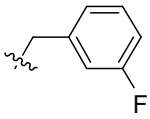
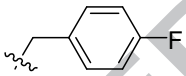
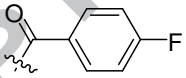
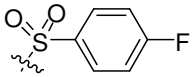
Table2 Synthesized derivatives **26-29**, **32** and their respective CCR5 calcium mobilization antagonism.

Table 3 Synthesized derivatives **33-36** and their respective CCR5 calcium mobilization antagonism.

Table 4 Antiviral activity of selected compounds.

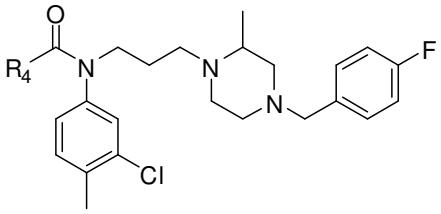
Table 1

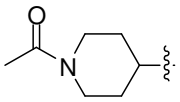
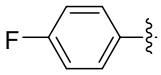
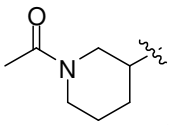
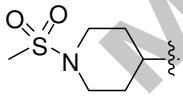
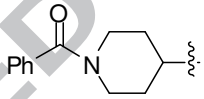


Compd	R_1	R_2	R_3	IC_{50} (nM) [a]
13		Cl	CH_3	8.3±2.3
14		Cl	CH_3	15.1±5.7
15		Cl	CH_3	25.1±6.1
16		Cl	CH_3	6.6±2.4
17		Cl	CH_3	30.1±10.1
18		Cl	CH_3	11.7±2.1
19		H	H	319.9±54.73
20		Cl	H	25.1±3.8
21		Cl	CH_3	30.0±4.8
22		Cl	CH_3	10.9±4.2
Maraviroc	-			13.1±2.7

[a] Values are means mean ±S.E.M. of three of separate experiments

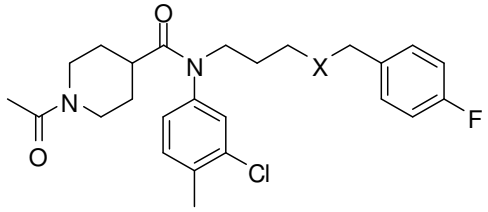
Table 2

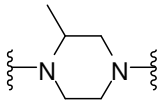
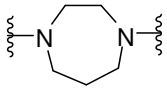
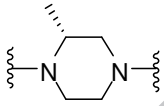
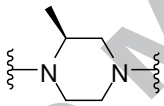


Compd	R_4	IC_{50} (nM) [a]
16		6.6±2.5
26		2158.0±523.9
27		204.1±52.6
28		259.7±92.9
29		17.1±1.7
32		33.2±7.2
Maraviroc	-	13.1±2.7

[a] Values are means mean ±S.E.M. of three of separate experiments

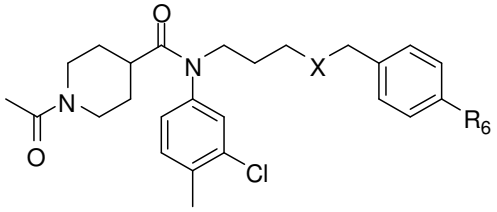
Table 3

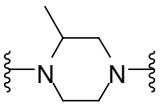
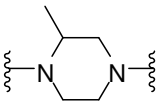
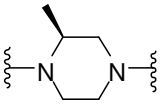


Compd	X	IC_{50} (nM) [a]
16		6.6±2.5
33		8.8±1.9
34		906.0±102.5
35		49.9±10.3
36		3.0±1.1
Maraviroc	-	13.1±2.7

[a] Values are means mean ±S.E.M. of three of separate experiments

Table 4



Compd	X	R_6	IC_{50} (nM)[a]
13		CN	47.2
16		F	140.6
33		F	31.4
36		F	75.1
Maraviroc			5.5

Graphical abstract