Synthesis and biological evaluation of novel *n*-[3-(4-phenylpip-erazin-1-yl)-propyl]-carboxamide derivatives

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A series of novel *N*-[3-(4-phenylpiperazin-1-yl)-propyl]-carboxamide derivatives were synthesised and studied for the potential treatment of HIV. These compounds were obtained through the efficient synthetic route that involved microwave assisted synthesis. These new compounds have been characterised by IR, ^{1}H NMR, MS and elemental analysis. The cell–cell fusion inhibitory activities of the compounds have also been evaluated.

Keywords: *N*-[3-(4-phenylpiperazin-1-yl)-propyl]-carboxamide, HIV, cell–cell fusion assays

AIDS is a disease of the human immune system caused by the human immunodeficiency virus (HIV). Despite the impressive role shown by highly active antiretroviral therapy (HAART), many patients are confronted with incomplete efficacy, toxicity, and the eventual emergence of a resistant virus.[1] Therefore, there is an urgent need to discover potent antiretroviral agents with novel mechanisms of action.

Discovery of the chemokine receptor R5 (CCR5) as a co-receptor for HIV-1 entry revealed a novel approach to HIV-1 epidemic prevention and treatment. Over the past decade, there has been an increased effort in the pharmaceutical industry to develop CCR5 antagonists. As shown in Fig. 1, these efforts have resulted in the FDA approval of the first small-molecule CCR5 antagonist, Maraviroc (UK-427,857, **1**) in 2007.[2] Takeda also disclosed TAK-220 (**2**) in their efforts toward a clinical application of a CCR5 antagonist,[3,4] which is in Phase I clinic trial now.

Condru reported that these two CCR5 antagonists seem to share a common binding site and share certain interactions through a molecular interaction study. By using site-directed mutagenesis and CCR5 homology modeling, it is believed that one of the most important features of these two compounds is showing strong salt-bridge interaction with Glu283 via their central basic nitrogen. The interaction between two compounds and Ile198 is primarily hydrophobic in nature while the interaction between Trp86 and these compounds involves T-shaped π–π stacking[5].

In view of these findings, we designed a chemical scaffold that combined the attractive characteristics of these two CCR5 antagonists through fragment assembly. We now report the synthesis and biological evaluation with cell–cell fusion (CCF) assays of a series of novel *N*-[3-(4-phenylpiperazin-1-yl)-propyl]-carboxamide derivatives **3a–j** which contain the central basic nitrogen in the piperazine ring and the hydrophobic aromatic ring, with our primary interest in finding novel compounds as potential treatments for HIV.

Results and discussion

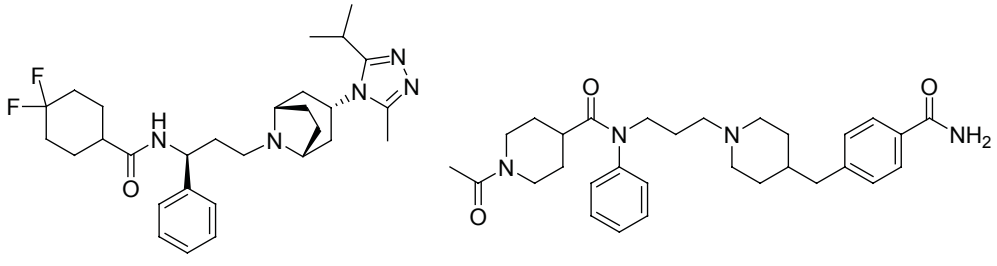
The synthetic routes to the target *N*-[3-(4-phenylpiperazin-1-yl)-propyl]-carboxamide derivatives **3a–j** are outlined in Scheme 1. Reaction of the appropriate anilines **4a** and **4b** with 1-bromo-3-chloropropane in acetonitrile under microwave irradiation in the presence of KI afforded compounds **5a** and **5b** in 67 and 65% yield, respectively. Acylation of **5a** and **5b** with compounds **6a–e** gave the chlorides **7a–f**. Diethanolamine **8** reacted with $SOCl_2$ in reflux $CHCl_3$ to afford nitrogen mustard hydrochloride **9**, which then was heated at 130 °C with different substituted anilines **10a–d** for 24 h in diethylene glycol monomethyl ether to give piperazine hydrochlorides **11a–d** with the yield from 52 to 75%. The chlorides **7a–f** were reacted with 4-substituted piperazine **11a–e** in the presence of KI and K_2CO_3 to afford the target compounds **3a–j**. The structures of the synthesised target compounds **3a–j** were confirmed by IR, ^{1}H NMR, MS and elemental analyses.

These compounds **3a–j** were screened for their cell–cell fusion inhibitory activity at 10 μM against target cells-effector cells. Compounds **3g** and **3h** showed 27%, 30% inhibition at 10 μM, respectively. But other compounds exhibited no inhibitory activity.

In summary, we have designed and synthesised a series of novel *N*-[3-(4-phenylpiperazin-1-yl)-propyl]-carboxamide derivatives through the efficient synthetic route and studied their cell–cell fusion inhibitory activity. Some tested compounds showed moderate inhibitory activity.

Experimental

Melting points were obtained on a Buchi B-540 apparatus (Buchi Labortechnik, Flawil, Switzerland) and are uncorrected. IR spectra were recorded on a Bruker VECTOR 22 FT-IR spectrophotometer. All ^{1}H NMR spectra were recorded on Bruker 500 MHz-spectrometer (Bruker Bioscience, Billerica, MA, USA) with $SiMe_4$ as the internal standard in $CDCl_3$ or DMSO-d_6. Chemical shifts were reported in δ values (ppm), relative to internal TMS, and J values were reported in



Maraviroc **1** TAK-220 **2**

Fig. 1

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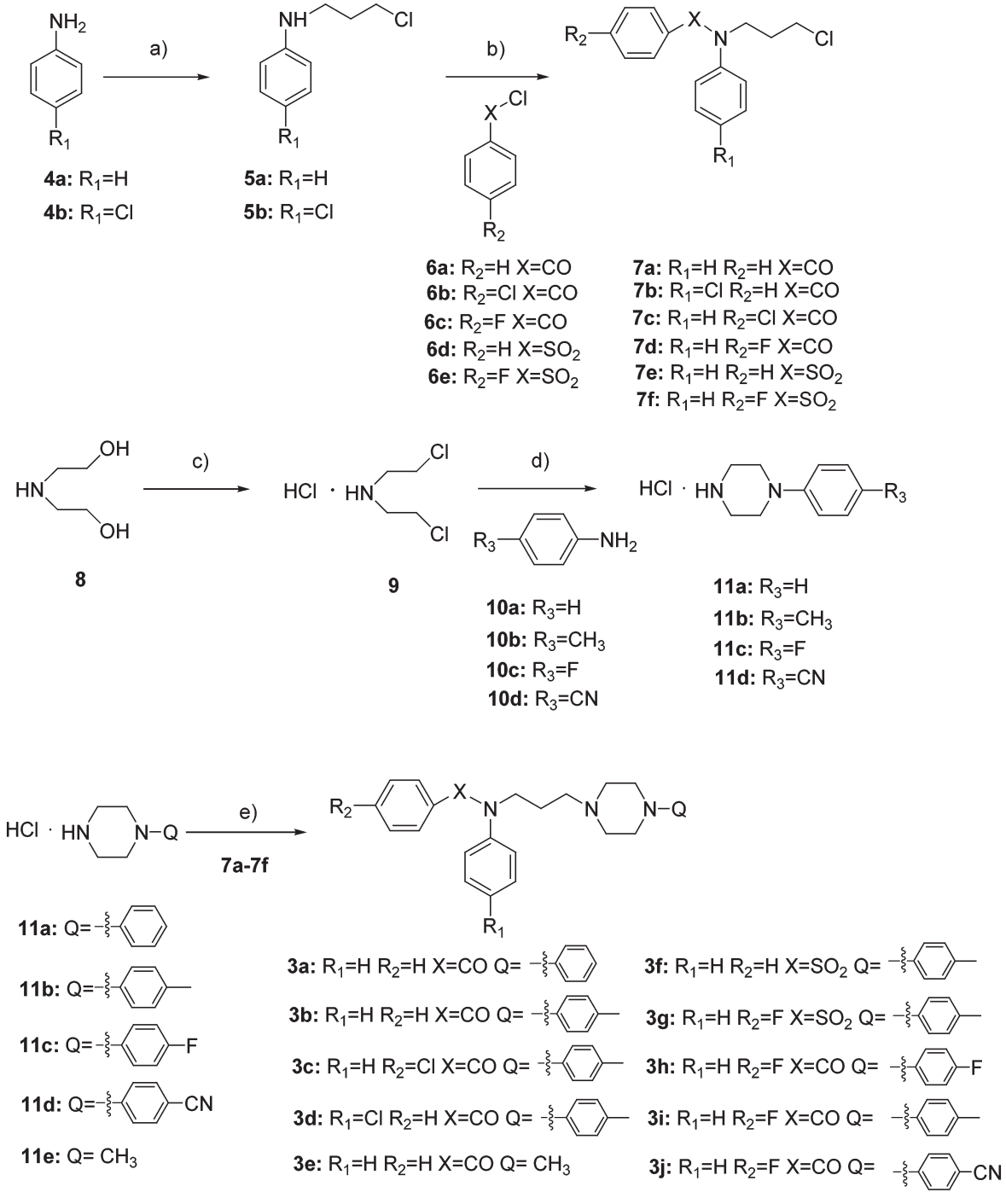

Reagents and conditions: a) $Br(CH_2)_3Cl$, KI, CH_3CN, MWI, 15min; b) Et_3N, DCM, 0°C, 5h; c) $SOCl_2$, $CHCl_3$, rt, 1h, reflux, 4h; d) EGME, 130°C, 24h; e) KI, K_2CO_3, CH_3CN, reflux, 24h.

Scheme 1

Hertz (Hz). Mass spectra (ESI, positive ion) were recorded on an Esquire-LC-00075 spectrometer (Bruker Bioscience). Element analyses were performed on an EA-1110 instrument. Reagents and solvents were of commercial quality, which are purchased from known commercial suppliers and were used without further purification.

Synthesis of compounds **5a–b***; typical procedure*

The appropriate aniline **4a–b** (9 mmol), 1-bromo-3-chloropropane (485 mg, 3 mmol), and KI (51 mg, 0.3 mmol) in CH_3CN (5 mL) was kept under stirring at 110 °C for 15 min using a Biotage microwave reactor. After cooling to room temperature, the mixture was filtered, and the filtrate was concentrated *in vacuo*. The residue was diluted with EtOAc (30 mL), washed with water (30 mL) and brine (3 × 15 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (petroleum ether:ethyl acetate = 12:1) to afford the products **5a–b**.

N-(3-Chloropropyl)-benzenamine (**5a**): Yield: 67%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.36–7.18 (m, 2H, ArH), 7.16–6.99 (m, 3H, ArH), 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-Chloro-N-(3-chloropropyl)-benzenamine (**5b**): Yield: 65%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.33–7.20 (m, 2H, ArH), 7.14–6.96 (m, 2H, ArH), 3.68–3.64 (m, 2H, CH_2), 3.58–3.53 (m, 2H, CH_2), 3.02–2.98 (m, 2H, CH_2). ESI-MS *m/z*: 204 $[M+H]^+$.

Synthesis of compounds **7a–f***; typical procedure*

To an ice-cooled stirred suspension of compound **5a–b** (1 mmol) in DCM (3 mL), was added Et_3N (0.4 mL, 3 mmol) followed by compound **6a–e** (1.2 mmol), and the mixture was stirred at 0 °C for 5 h. The mixture was diluted with saturated aqueous $NaHCO_3$ (10 mL), and the organic layer was separated. The aqueous layer was extracted with DCM (10 mL), and the combined organic layer was washed with brine (2 × 10 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate=10:1) to afford the product **7a–f**.

N-(3-Chloropropyl)-N-phenylbenzamide (**7a**): Yield: 85%; yellow oil; IR (film, cm^{-1}): 1650 (C=O); ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.29–7.18 (m, 5H, ArH), 7.13 (t, J = 7.5 Hz, 3H, ArH), 7.03 (d, J = 8.0 Hz, 2H, ArH), 4.06 (t, J = 7.5 Hz, 2H, CH_2), 3.60 (t, J = 6.5 Hz, 2H, CH_2), 2.19–2.15 (m, 2H, CH_2). ESI-MS *m/z*: 274 $[M+H]^+$.

N-(4-Chlorophenyl)-N-(3-chloropropyl)-benzamide (**7b**): Yield: 88%; yellow oil; IR (film, cm^{-1}): 1684 (C=O); ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.33 (d, J = 8.0 Hz, 3H, ArH), 7.24–7.28 (m, 4H, ArH), 7.00 (d, J = 8.5 Hz, 2H, ArH), 4.16 (t, J = 6.5 Hz, 2H, CH_2), 3.71 (t, J = 6.5 Hz, 2H, CH_2), 1.79–1.74 (m, 2H, CH_2). ESI-MS *m/z*: 308 $[M+H]^+$.

4-Chloro-N-(3-chloropropyl)-N-phenylbenzamide (**7c**): Yield: 83%; yellow oil; IR (film, cm^{-1}): 1662 (C=O); ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.26–7.13 (m, 5H, ArH), 7.11 (d, J = 8.5 Hz, 2H, ArH), 7.01 (d, J = 8.0 Hz, 2H, ArH), 4.04 (t, J = 7.5 Hz, 2H, CH_2), 3.59 (t, J = 7.0 Hz, 2H, CH_2), 2.17–2.12 (m, 2H, CH_2). ESI-MS *m/z*: 308 $[M+H]^+$.

N-(3-Chloropropyl)-4-fluoro-N-phenylbenzamide (**7d**): Yield: 90%; white solid; m.p. 71–73 °C; IR (KBr, cm^{-1}): 1675 (C=O); ^1H NMR ($CDCl_3$, 500 MHz) δ : 7.30–7.06 (m, 5H, ArH), 7.01–6.99 (m, 2H, ArH), 6.82–6.79 (m, 2H, ArH), 4.05 (t, J = 7.0 Hz, 2H, CH_2), 3.60 (t, J = 7.0 Hz, 2H, CH_2), 2.18–2.13 (m, 2H, CH_2). ESI-MS *m/z*: 292 $[M+H]^+$.

N-(3-Chloropropyl)-N-phenylbenzenesulfonamide (**7e**): Yield: 89%; white oil; IR (film, cm^{-1}): 1312 and 1152 (N–SO_2); ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.60–7.56 (m, 3H, ArH), 7.48–7.45 (m, 2H, ArH), 7.33–7.30 (m, 3H, ArH), 7.04–7.02 (m, 2H, ArH), 3.69 (t, J = 7.0 Hz, 2H, CH_2), 3.57 (t, J = 7.0 Hz, 2H, CH_2), 1.94–1.90 (m, 2H, CH_2). ESI-MS *m/z*: 310 $[M+H]^+$.

N-(3-Chloropropyl)-4-fluoro-N-phenylbenzenesulfonamide (**7f**): Yield: 90%; white solid; m.p. 70-72 °C; IR (KBr, cm^{-1}): 1345 and 1167 (N-SO_2); ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.60–7.57 (m, 2H, ArH), 7.34–7.32 (m, 3H, ArH), 7.16–7.12 (m, 2H, ArH), 7.05–7.03 (m, 2H, ArH), 3.69 (t, J = 7.0 Hz, 2H, CH_2), 3.57 (t, J = 7.0 Hz, 2H, CH_2), 1.95–1.90 (m, 2H, CH_2). ESI-MS *m/z*: 328 $[M+H]^+$.

Synthesis of Bis-(2-chloroethyl)-amine hydrochloride (**9**): To a solution of diethanolamine **8** (1.05 g, 10 mmol) in $CHCl_3$ (2 mL), $SOCl_2$ (15 mmol) in $CHCl_3$ (3 mL) was added dropwise at 0 °C, then the mixture was stirred at room temperature for 1 h, and refluxed for 4 h. After evaporation of the excessive $SOCl_2$, the residue was crystallised from ethanol to give 1.13 g (80%) of compound **9** as a white solid. m.p. 212–215 °C. [lit[6] m.p. 212–216 °C].

*Synthesis of compounds (***11a–d***);*[7] *typical procedure*

A mixture of the appropriate aniline **10a–d** (4.0 mmol), bis-(2-chloroethyl)-amine hydrochloride **9** (0.73 g, 4.0 mmol), and diethylene glycol monomethyl ether (1 mL) was heated at 130 °C for 24 h under N_2. After cooling to room temperature, the mixture was dissolved in MeOH (4 mL) followed by addition of Et_2O (150 mL). The precipitate was filtered, washed with Et_2O and recrystallised from ethanol to give **11a–d**.

1-Phenylpiperazine hydrochloride (**11a**): Yield: 71%; white solid; m.p. 156–158 °C. [lit.[8] m.p.157–158 °C].

1-p-Tolylpiperazine hydrochloride (**11b**): Yield: 75%; white solid; m.p. 179–182 °C. [lit[8] m.p.175–178 °C].

1-(4-Fluorophenyl)-piperazine hydrochloride (**11c**): Yield: 65%; white solid; m.p. 182–185 °C. [lit[9] m.p. 180–184 °C].

4-(Piperazin-1-yl)-benzonitrile hydrochloride (**11d**): Yield: 52%; yellow solid; m.p. 160 °C (decomp). [lit[10] m.p. 160 °C (decomp)].

*Synthesis of compound (***3a–j***); typical procedure*

A mixture of compound **7a–f** (0.5 mmol), compound **11a–e** (0.5 mmol), KI (83 mg, 0.5 mmol), and K_2CO_3 (208 mg, 1.5 mmol) in MeCN (8 mL) was refluxed for 24 h. After cooling to room temperature, the mixture was concentrated *in vacuo*, the residue was diluted with water (5 mL) and extracted with EtOAc (3 × 10 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate=1:1) to afford the product **3a–j**.

N-Phenyl-N-(3-(4-phenylpiperazin-1-yl)-propyl)-benzamide (**3a**): Yield: 52%; yellow oil; IR (film, cm^{-1}): 1643(C=O); ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.29–7.20 (m, 7H, ArH), 7.13 (t, J = 7.5 Hz, 3H, ArH), 7.03 (d, J = 8.0 Hz, 2H, ArH), 6.91 (d, J = 8.0 Hz, 2H, ArH), 6.83 (t, J = 8.0 Hz, 1H, ArH), 3.98 (t, J = 7.0 Hz, 2H, CH_2), 3.16 (t, J = 5.0 Hz, 4H, piperazinyl-H), 2.56 (t, J = 5.0 Hz, 4H, piperazinyl-H), 2.45 (t, J = 7.5 Hz, 2H, CH_2), 1.91–1.87 (m, 2H, CH_2). ESI-MS *m/z*: 400 $[M+H]^+$. Anal. Calcd for $C_{26}H_{29}N_3O$: C, 78.16; H, 7.32; N, 10.52. Found: C, 78.05; H, 7.39; N, 10.39%.

N-Phenyl-N-(3-(4-p-tolylpiperazin-1-yl)-propyl)-benzamide (**3b**): Yield: 54%; yellow solid; m.p. 60–62 °C; IR (KBr, cm^{-1}): 1655(C=O); ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.28 (d, J = 7.5 Hz, 2H, ArH), 7.19 (t, J = 7.5 Hz, 3H, ArH), 7.19 (t, J = 7.5 Hz, 3H, ArH), 7.03 (t, J = 10.0 Hz, 4H, ArH), 6.82 (d, J = 8.5 Hz, 2H, ArH), 3.98 (t, J = 7.5 Hz, 2H, CH_2), 3.11 (t, J = 4.5 Hz, 4H, piperazinyl-H), 2.55 (t, J = 4.5 Hz, 4H, piperazinyl-H), 2.45 (t, J = 7.0 Hz, 2H, CH_2), 2.26 (s, 3H, CH_3), 1.92–1.88 (m, 2H, CH_2). ESI-MS *m/z*: 414 $[M+H]^+$. Anal. Calcd for $C_{27}H_{31}N_3O$: C, 78.42; H, 7.56; N, 10.16. Found: C, 78.32; H, 7.65; N, 10.25%.

4-Chloro-N-phenyl-N-(3-(4-p-tolylpiperazin-1-yl)-propyl)-benzamide (**3c**): Yield: 60%; pale yellow solid; m.p. 112–113 °C; IR (KBr, cm^{-1}): 1665(C=O); ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.30–7.23 (m, 5H, ArH), 7.19–7.14 (m, 4H, ArH), 7.00 (d, J = 8.5 Hz, 2H, ArH), 6.79 (d, J = 8.5 Hz, 2H, ArH), 3.86 (t, J = 6.5 Hz, 2H, CH_2), 2.99 (t, J = 4.5 Hz, 4H, piperazinyl-H), 2.41 (t, J = 4.5 Hz, 4H, piperazinyl-H), 2.33 (t, J = 7.0 Hz, 2H, CH_2), 2.22 (s, 3H, CH_3), 1.74–1.69 (m, 2H, CH_2). ESI-MS *m/z*: 448 $[M+H]^+$. Anal. Calcd for $C_{27}H_{30}ClN_3O$: C, 72.39; H, 6.75; N, 9.38. Found: C, 72.45; H, 6.70; N, 9.40%.

N-(4-Chlorophenyl)-N-(3-(4-p-tolylpiperazin-1-yl)-propyl)-benzamide (**3d**): Yield: 53%; yellow solid; m.p. 90–92 °C; IR (KBr, cm^{-1}): 1680(C=O); ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.28–7.24 (m, 2H, ArH), 7.20–7.18 (m, 4H, ArH), 7.05 (d, J = 8.5 Hz, 2H, ArH), 6.97 (d, J = 8.5 Hz, 2H, ArH), 6.82 (d, J = 8.5 Hz, 2H, ArH), 3.95 (t, J = 7.5 Hz, 2H, CH_2), 3.11 (t, J = 5.0 Hz, 4H, piperazinyl-H), 2.54 (t, J = 5.0 Hz, 4H, piperazinyl-H), 2.44 (t, J = 7.0 Hz, 2H, CH_2), 2.26 (s, 3H, CH_3), 1.91–1.86 (m, 2H, CH_2). ESI-MS *m/z*: 448 $[M+H]^+$. Anal. Calcd for $C_{27}H_{30}ClN_3O$: C, 72.39; H, 6.75; N, 9.38. Found: C, 72.49; H, 6.67; N, 9.44%.

N-(3-(4-Methylpiperazin-1-yl)-propyl)-N-phenylbenzamide (**3e**): Yield: 65%; yellow solid; m.p. 45–47 °C; IR (KBr, cm^{-1}): 1640(C=O); ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.26 (d, J = 8.5 Hz, 2H, ArH), 7.20 (t, J = 8.5 Hz, 3H, ArH), 7.13 (t, J = 8.0 Hz, 3H, ArH), 7.02 (d, J = 8.0 Hz, 2H, ArH), 3.95 (t, J = 7.5 Hz, 2H, CH_2), 2.48–2.40 (m, 10H, CH_2 and piperazinyl-H), 2.09 (s, 3H, CH_3), 1.87–1.83 (m, 2H, CH_2). ESI-MS *m/z*: 338 $[M+H]^+$. Anal. Calcd for $C_{21}H_{27}N_3O$: C, 74.74; H, 8.06; N, 12.45. Found: C, 74.69; H, 8.01; N, 12.40%.

N-Phenyl-N-(3-(4-p-tolylpiperazin-1-yl)-propyl)-benzenesulfonamide (**3f**): Yield: 58%; yellow solid; m.p. 113–116 °C; IR (KBr, cm^{-1}): 1320 and 1150 (N-SO_2); ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.57 (t, J = 7.0 Hz, 3H, ArH), 7.44 (t, J = 7.5 Hz, 2H, ArH), 7.33–7.30 (m, 3H, ArH), 7.07–7.04 (m, 4H, ArH), 6.82 (d, J = 8.5 Hz, 2H, ArH), 3.61 (t, J = 7.5 Hz, 2H, CH_2), 3.07 (t, J = 5.0 Hz, 4H, piperazinyl-H), 2.50 (t, J = 5.0 Hz, 4H, piperazinyl-H), 2.43 (t, J = 7.0 Hz, 2H, CH_2), 2.27 (s, 3H, CH_3), 1.70–1.66 (m, 2H, CH_2). ESI-MS *m/z*: 450 $[M+H]^+$. Anal. Calcd for $C_{26}H_{31}N_3O_2S$: C, 69.42; H, 6.95; N, 9.35. Found: C, 69.38; H, 6.99; N, 9.40%.

4-Fluoro-N-phenyl-N-(3-(4-p-tolylpiperazin-1-yl)-propyl)-benzenesulfonamide (**3g**): Yield: 55%; yellow solid; m.p. 100–102 °C; IR (KBr, cm^{-1}): 1340 and 1170 (N–SO_2); ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.60–7.57 (m, 2H, ArH), 7.33–7.31 (m, 3H, ArH), 7.11 (t, J = 8.5 Hz, 2H, ArH), 7.06–7.03 (m, 4H, ArH), 6.82–6.80 (m, 2H, ArH), 3.57 (t, J = 7.5 Hz, 2H, CH_2), 3.07 (t, J = 5.0 Hz, 4H, piperazinyl-H), 2.50 (t, J = 5.0 Hz, 4H, piperazinyl-H), 2.42 (t, J=7.0 Hz, 2H, CH_2), 2.26 (s, 3H, CH_3), 1.69–1.65 (m, 2H, CH_2). ESI-MS *m/z*: 468 $[M+H]^+$. Anal. Calcd for $C_{26}H_{30}FN_3O_2S$: C, 66.78; H, 6.47; N, 8.99. Found: C, 66.68; H, 6.49; N, 8.93%.

4-Fluoro-N-(3-(4-(4-fluorophenyl)-piperazin-1-yl)-propyl)-N-phenylbenzamide (**3h**): Yield: 44%; yellow solid; m.p. 74–77 °C; IR (KBr, cm^{-1}): 1670(C=O); ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.31–7.16 (m, 5H, ArH), 7.02 (d, J = 7.5 Hz, 2H, ArH), 6.93 (t, J = 8.5 Hz, 2H, ArH), 6.82–6.87 (m, 4H, ArH), 3.97 (t, J = 7.5 Hz, 2H, CH_2), 3.09 (t, J = 5.0 Hz, 4H, piperazinyl-H), 2.57 (t, J = 5.0 Hz, 4H, piperazinyl-H), 2.45 (t, J = 7.0 Hz, 2H, CH_2), 1.92–1.87 (m, 2H, CH_2). ESI-MS

m/z: 436 $[M+H]^+$. Anal. Calcd for $C_{26}H_{27}F_2N_3O$: C, 71.70; H, 6.25; N, 9.65. Found: C, 71.65; H, 6.29; N, 9.67%.

4-Fluoro-N-phenyl-N-(3-(4-p-tolylpiperazin-1-yl)-propyl)-benzamide (**3i**): Yield: 53%; yellow solid; m.p. 70–72 °C; IR (KBr, cm^{-1}): 1663(C=O); ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.33–7.17 (m, 5H, ArH), 7.05 (d, J = 7.5 Hz, 2H, ArH), 7.01 (t, J = 7.5 Hz, 2H, ArH), 6.82–6.86 (m, 4H, ArH), 3.98 (t, J = 7.5 Hz, 2H, CH_2), 3.13 (t, J = 5.0 Hz, 4H, piperazinyl-H), 2.57 (t, J = 5.0 Hz, 4H, piperazinyl-H), 2.47 (t, J = 7.0 Hz, 2H, CH_2), 2.27 (s, 3H, CH_3), 1.74–1.70 (m, 2H, CH_2). ESI-MS *m/z*: 432 $[M+H]^+$. Anal. Calcd for $C_{27}H_{30}FN_3O$: C, 75.15; H, 7.01; N, 9.74. Found: C, 75.20; H, 6.99; N, 9.71%.

N-(3-(4-(4-cyanophenyl)-piperazin-1-yl)-propyl)-4-fluoro-N-phenylbenzamide (**3j**): Yield: 41%; yellow solid; m.p. 82–84 °C; IR (KBr, cm^{-1}): 1655(C=O), 2218(CN); ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.48 (d, J = 4.0 Hz, 2H, ArH), 7.31–7.16 (m, 5H, ArH), 7.02 (d, J = 7.5 Hz, 2H, ArH), 6.82–6.86 (m, 4H, ArH), 3.98 (t, J = 7.5 Hz, 2H, CH_2), 3.29 (t, J = 5.0 Hz, 4H, piperazinyl-H), 2.53 (t, J = 5.0 Hz, 4H, piperazinyl-H), 2.44 (t, J = 7.0 Hz, 2H, CH_2), 1.78-1.74 (m, 2H, CH_2). ESI-MS *m/z*: 443 $[M+H]^+$. Anal. Calcd for $C_{27}H_{27}FN_4O$: C, 73.28; H, 6.15; N, 12.66. Found: C, 73.35; H, 6.20; N, 12.63%.

Biological assays

Effector cell line: This cell line express HIV envelope protein gp160 and chimera protein Rn-Dn. Rn-Dn consists of the N terminal of renilla luciferase and the N terminal of DnaE intein from Anacystis nidulans R2 PCC7942.

Target cell line: This cell line express chemokine receptor 5(CCR5), CD4 protein and chimera protein Dc-Rc. Dc-Rc consists of the C teminal of renilla luceferase and the C teminal of NnaE intein from Anacystis nidulans R2 PCC7942.

Cell–cell fusion assays (CCF assays)

The effector cells were plated in 24 well white culture plates at 7.5×10^4 cell per well in DMEM supplemented with 10% FBS, 800 μg mL^{-1} G418. The target cells in the growth medium were then added to the plates at 7.5×10^4 cells/50 μL/well and incubated for 5 h. At the end of coculture, 70 μL of renilla luceferase assay lysis was added into each well, and the cultures were gently shaken for 15 min. At the same time, 20 μL of Renilla Luciferase Assay Reagent was added to the luminometer tube and then 20 μL of cell lysate added to the tube. Alter mixing quickly, the tube was flicked for 1 second and then placed in a FB12 luminometer to permit measurements. Luminescence was integrated over 1 second with a 2-second delay. When small molecule compounds needed to be added to the CCF assay system, the compounds were diluted manually in DMSO. Then, 10 μL of the diluted compounds was added to the effector cells just before the addition of target cells, thus making the final concentration of DMSO in the coculture 0.5%.

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References

1 S. Yerly, L. Kaiser, E. Race, J.P. Bru, F. Clavel and L. Perrin, *Lancet*, 1999, **354**, 729.
2 D.M. Rodger and M.N. Richard, *Clin. Infect. Dis.*, 2008, **47**, 236.
3 K. Takashima, H. Miyake, N. Kanzaki, Y. Tagawa, X. Wang, Y. Sugihara, Y. Iizawa and M. Baba, *Antimicrob. Agents. Chemother.*, 2005, **49**, 3474.
4 M. Nishikawa, K. Takashima, T. Nishi, R.A. Furuta, N. Kanzaki, Y. Yamamoto and J. Fujisawa, *Antimicrob. Agents. Chemother.*, 2005, **49**, 4708.
5 R. Kondru, J. Zhang, C.H. Ji, T. Mirzadegan, D. Rotstein, S. Sankuratri and M. Dioszegi, *Mol. Pharmacol.*, 2008, **73**, 789.
6 S. Xu, W. Li and K.C. Chen, *Chinese. J. Chem.*, 2007, **25**, 778.
7 G.L. Kevin and J.R. Albert, *Tetrahedron. Lett.*, 2005, **46**, 7921.
8 B.S. Wagh, B.P. Patil, M.S. Jain, S.S. Harak and S.B. Wagh, *Heterocycl. Commun.*, 2007, **13**, 165.
9 X. Chen, L. Wang, Z.Z. Zhao, X.Q. Zhang and X.H. Chen, *Acta. Pharm. Sin.*, 2002, **37**, 343.
10 D.A. Walsh and J.M. Yanni, EP 269383, 1988.