Supporting Information

Increasing selectivity of CC chemokine receptor 8 antagonists by engineering non-desolvation related interactions with the intended and off-target binding sites

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Synthesis details

General procedures

All starting materials and chemical reagents were purchased from commercial suppliers and used without further purification, unless otherwise indicated. Preparative HPLC was carried out on Gilson Liquid Chromatography Preparative System using Chiralpak C-18 column 25x10 cm. NMR spectra were measured at 300 MHz or 400 MHz on Varian 300 and Varian 400. Chemical shifts are reported in ppm downfiled from TMS internal standard. Mass Spectra (MS) were measured on HP 5890 GC-MS spectrometer and on HP 1100 HPLC-MS spectrometer. Purity of compounds was determined by HPLC with either of methods A-C, and purity of the target compounds was ≥ 95 %. Detection was carried out at 220, 254 and 280 nm.

HPLC Method A was performed with an Agilent 1100 series machine on Kromasil© C18 5µm 3.0x100mm column. The aqueous phase was water/TFA (99.8/0.1) and the organic phase was acetonitrile/TFA (99.92/0.08). Flow was 1 ml/min and the gradient was set from 10 to 100% of organic phase over 20 min.

HPLC Method B was performed with an Agilent 1100 series machine on XTerra® RP_8 5µm 3.0x100mm column. The aqueous phase was 15 mM NH_3 in water and the organic phase was acetonitrile. Flow was 1 ml/min and the gradient was set from 10 to 100% of organic phase over 20 min.

HPLC Method C was performed with an Agilent 1100 series machine on BDS C-18 5µm 4.6 x 250mm column. The aqueous phase was 20 mM NH_4OAc in water and the organic phase was acetonitrile. Flow was 0.7 ml/min and the gradient was set from 50 to 100% of organic phase over 10 min.

General method for reductive amination:

The spiro amine (1 eq), aldehyde (1.2 eq), $NaBH(OAc)_3$ (2 eq) and a catalytic amount of AcOH were mixed in dichloromethane and stirred over night at room temperature. The mixture was diluted with dichloromethane and washed with sat. aqueous $NaHCO_3$-solution, water and dried over Na_2SO_4. The solvents were removed *in vacuo* and the crude product was purified using acidic ion-exchange resin or by column chromatography on silica eluting with dichloromethane/MeOH/NH_3 (aq).

General method for Boc-deprotection:

The boc-protected amine was dissolved in MeOH and methanolic HCl (formed by adding acetyl chloride to cold MeOH) was added. The mixture was stirred at room temperature for 3 h after which the solvents were removed *in vacuo*. The solid residue was either recrystallised from Et_2O providing a HCl salt, or purified using ion-exchange resin, providing the free base.

General method for amide coupling:

A 0.055 mM mixture of the spiro amine (1 eq), HATU (1 eq), the appropriate acid (1.2 eq) and triethylamine (1.8 eq) in dichloromethane was stirred at room temperature for 1.5 h. The reaction mixture was diluted with EtOAc and washed with sodium hydrogen carbonate solution. The organic layer was isolated, evaporated to dryness and the residue was purified by preparative HPLC (RP-18) eluting with CH_3CN and water with 0.1% TFA to give the products as white solids.

Characterization data

3-(4-Chlorobenzoyl)-9-(3-phenoxybenzyl)-3,9-diazaspiro[5.5]undecane trifluoroacetate (1). ^{1}H NMR (400 MHz, CD_3OD) δ 7.51 - 7.44 (m, 3H), 7.43 - 7.36 (m, 4H), 7.26 - 7.08 (m, 5H), 7.04 (d, *J* = 7.7 Hz, 1H), 4.30 (s, 2H), 3.74 (s, 2H), 3.46 - 3.30 (m, 4H), 3.25 - 3.04 (m, 2H), 2.02 (d, *J* = 14.4 Hz, 2H), 1.85 - 1.38 (m, 6H). LC-MS: m/z 475/477 3:1 [MH^+].

3-(4-Chlorobenzoyl)-9-[2-(2-methoxyphenoxy)benzyl]-3,9-diazaspiro[5.5]undecane trifluoroacetate (3). ^{1}H NMR (400 MHz, CD_3OD) δ 7.55 - 7.26 (m, 7H), 7.21 - 7.01 (m, 4H), 6.61 (d, J = 9.0 Hz, 1H), 4.53 (s, 2H), 3.75 (s, 5H), 3.56 - 3.48 (m, 2H), 3.47 - 3.39 (m, 2H), 3.31 - 3.22 (m, 2H), 2.06 (d, J = 13.9 Hz, 2H), 1.87 - 1.40 (m, 6H). LC-MS: m/z 505/507 3:1 [MH^+].

3-[2-(2-Methylpropoxy)benzyl]-9-(pyridin-4-ylcarbonyl)-3,9-diazaspiro[5.5]undecane trifluoroacetate (5). ^{1}H NMR (400 MHz, CD_3OD) δ 9.00 – 8.86 (m, 2H), 8.12 – 8.05 (m, 2H), 7.51 – 7.42 (m, 2H), 7.16 – 7.10 (m, 1H), 7.09 - 6.99 (m, 1H), 4.38 (d, 2H), 3.95 - 3.87 (m, 2H), 3.82 - 3.75 (m, 2H), 3.47 - 3.33 (m, 4H), 3.29 - 3.17 (m, 2H), 2.21 – 2.09 (m, 1H), 2.05 (d, 2H) 1.83 - 1.42 (m, 6H), 1.15 - 1.04 (m, 6H). LC-MS: m/z 423 [MH^+].

3-[2-(2-Methoxyphenoxy)benzyl]-9-(pyridin-4-ylcarbonyl)-3,9-diazaspiro[5.5]undecane trifluoroacetate (8). ^{1}H NMR (300 MHz, DMSO-d^6) δ 9.34 - 9.09 (m, 1H), 8.71 - 8.67 (m, 2H), 7.56 (d, *J* = 7.5 Hz, 1H), 7.42 (d, *J* = 5.3 Hz, 2H), 7.39 - 6.99 (m, 6H), 6.56 - 6.48 (m, 1H), 4.51 - 4.41 (m, 2H), 3.74 - 3.68 (m, 3H), 3.66 - 3.57 (m, 2H), 3.42 - 3.31 (m, 2H), 3.29 - 3.07 (m, 4H), 1.98 - 1.87 (m, 2H), 1.76 - 1.27 (m, 6H). LC-MS: m/z 473 [MH^+].

3-[2-(2-Methoxyphenoxy)benzyl]-9-(pyrimidin-4-ylcarbonyl)-3,9-diazaspiro[5.5]undecane trifluoroacetate (10). ^{1}H NMR (400 MHz, DMSO-d^6) δ 9.28 - 9.16 (m, 2H), 8.96 (d, *J* = 5.1 Hz, 1H), 7.66 - 7.63 (m, 1H), 7.59 - 7.54 (m, 1H), 7.38 - 7.26 (m, 2H), 7.25 - 7.09 (m, 3H), 7.09 - 7.01 (m, 1H), 6.52 (t, *J* = 7.4 Hz, 1H), 4.50 - 4.43 (m, 2H), 3.71 (d, *J* = 7.9 Hz, 3H), 3.67 - 3.61 (m, 2H), 3.40 - 3.32 (m, 2H), 3.32 - 3.09 (m, 4H), 1.98 - 1.90 (m, 2H), 1.75 - 1.51 (m, 4H), 1.49 - 1.31 (m, 2H). LC-MS: m/z 474 [MH$^+$].

3-(3-Phenoxybenzyl)-9-(pyridin-4-ylcarbonyl)-3,9-diazaspiro[5.5]undecane trifluoroacetate (11). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.97 (s, 1H), 8.91 - 8.85 (m, 2H), 7.75 - 7.65 (m, 2H), 7.46 - 7.30 (m, 4H), 7.24 - 6.94 (m, 5H), 4.16 (s, 2H), 3.49 - 3.38 (m, 2H), 3.35 - 3.23 (m, 2H), 3.04 - 2.93 (m, 2H), 2.89 - 2.72 (m, 2H), 2.30 - 2.14 (m, 2H), 1.91 - 1.77 (m, 2H), 1.74 - 1.43 (m, 4H). LC-MS: m/z 443 [MH$^+$].

3-(3-Phenoxybenzyl)-9-(pyrimidin-4-ylcarbonyl)-3,9-diazaspiro[5.5]undecane trifluoroacetate (12). ^{1}H NMR (300 MHz, DMSO-d^6) δ 9.30 - 9.16 (m, 1H), 9.02 - 8.93 (m, 1H), 7.69 - 7.62 (m, 1H), 7.56 - 7.37 (m, 3H), 7.29 - 7.02 (m, 6H), 4.38 - 4.25 (m, 2H), 3.73 - 3.35 (m, 2H), 3.34 - 2.96 (m, 6H), 2.01 - 1.81 (m, 2H), 1.75 - 1.28 (m, 6H). LC-MS: m/z 444 [MH$^+$].

3-[3-(2-Methoxyphenoxy)benzyl]-9-(pyrimidin-4-ylcarbonyl)-3,9-diazaspiro[5.5]undecane trifluoroacetate (13). ^{1}H NMR (400 MHz, DMSO-d^6) δ 9.40 - 9.28 (m, 1H), 9.26 - 9.21 (m, 1H), 9.00 - 8.93 (m, 1H), 7.67 - 7.62 (m, 1H), 7.44 - 7.35 (m, 1H), 7.29 - 7.17 (m, 2H), 7.16 - 7.05 (m, 2H), 7.05 - 6.96 (m, 2H), 6.93 - 6.86 (m, 1H), 4.32 - 4.22 (m, 2H), 3.72 (d, *J* = 3.0 Hz, 3H), 3.68 - 3.59 (m, 2H), 3.33 - 3.24 (m,

2H), 3.21 - 3.12 (m, 2H), 3.12 - 2.94 (m, 2H), 1.97 - 1.85 (m, 2H), 1.73 - 1.64 (m, 1H), 1.62 - 1.41 (m, 4H), 1.39 - 1.30 (m, 1H). LC-MS: m/z 474 [MH^+].

3-(2-Phenoxybenzyl)-9-(pyrimidin-4-ylcarbonyl)-3,9-diazaspiro[5.5]undecane trifluoroacetate (15). ^{1}H NMR (400 MHz, CD_3OD) δ 9.23 - 9.20 (m, 1H), 8.98 - 8.92 (m, 1H), 7.66 - 7.62 (m, 1H), 7.61 - 7.55 (m, 1H), 7.49 - 7.39 (m, 3H), 7.27 - 7.18 (m, 2H), 7.14 - 7.05 (m, 2H), 6.92 - 6.84 (m, 1H), 4.50 - 4.44 (m, 2H), 3.80 - 3.74 (m, 2H), 3.52 - 3.41 (m, 4H), 3.35 - 3.19 (m, 2H), 2.11 - 2.02 (m, 2H), 1.85 - 1.80 (m, 1H), 1.77 - 1.63 (m, 3H), 1.62 - 1.57 (m, 1H), 1.55 - 1.49 (m, 1H). LC-MS: m/z 444 [MH^+] main signal 443 [M+].

3-[(2,2-Dimethyl-3,4-dihydro-2H-chromen-6-yl)methyl]-9-(pyrimidin-4-ylcarbonyl)-3,9-diazaspiro[5.5]undecane trifluoroacetate (17). ^{1}H NMR (400 MHz, CD_3OD) δ 9.23 - 9.20 (m, 1H), 8.94 (t, *J* = 5.2 Hz, 1H), 7.67 - 7.63 (m, 1H), 7.22 - 7.14 (m, 2H), 6.82 - 6.78 (m, 1H), 4.20 (d, *J* = 10.3 Hz, 2H), 3.81 - 3.74 (m, 2H), 3.48 - 3.40 (m, 2H), 3.38 - 3.32 (m, 2H), 3.21 - 3.03 (m, 2H), 2.86 - 2.79 (m, 2H), 2.09 - 1.99 (m, 2H), 1.88 - 1.47 (m, 8H), 1.33 (s, 3H), 1.32 (s, 3H). LC-MS: m/z 436 [MH^+] main signal 435 [M+].

4-({9-[(2,2-Dimethyl-2,3-dihydro-1-benzofuran-4-yl)methyl]-3,9-diazaspiro[5.5]undec-3-yl}carbonyl)pyridin-2-amine trifluoroacetate (25). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, *J* = 5.2 Hz, 1H), 7.06 (t, *J* = 8.0 Hz, 1H), 6.78 (d, *J* = 7.6 Hz, 1H), 6.66 - 6.57 (m, 2H), 6.46 (s, 1H), 5.31 (s, 1H), 4.53 (s, 2H), 3.72 - 3.65 (m, 2H), 3.42 - 3.39 (m, 2H), 3.33 - 3.28 (m, 2H), 3.01 (s, 2H), 2.41 - 2.36 (m, 4H), 1.63 - 1.51 (m, 6H), 1.48 (s, 6H), 1.44 - 1.38 (m, 2H). LC-MS: m/z 436 [MH^+] main signal 435 [M+].

6-({9-[(2,2-Dimethyl-3,4-dihydro-2H-chromen-6-yl)methyl]-3,9-diazaspiro[5.5]undec-3-yl}carbonyl)pyridin-3-amine trifluoroacetate (18). ^{1}H NMR

(300 MHz, CD_3OD) δ 7.96 (d, *J* = 2.5 Hz, 1H), 7.44 (d, *J* = 8.3 Hz, 1H), 7.26 - 7.13 (m, 3H), 6.80 (d, *J* = 8.2 Hz, 1H), 4.20 (s, 2H), 3.72 - 3.55 (m, 4H), 3.40 - 3.33 (m, 2H), 3.19 - 3.05 (m, 2H), 2.83 (t, *J* = 6.8 Hz, 2H), 2.07 - 1.96 (m, 2H), 1.84 (t, *J* = 6.7 Hz, 2H), 1.79 - 1.71 (m, 2H), 1.67 - 1.43 (m, 4H), 1.32 (s, 6H). LC-MS: m/z 450 [MH^+].

6-({9-[(2-*tert*-Butyl-2-methyl-1,3-benzodioxol-4-yl)methyl]-3,9-diazaspiro[5.5]undec-3-yl}carbonyl)pyridin-3-amine trifluoroacetate (28). ^1H NMR (400 MHz, CD_3OD) δ 7.96 (d, *J* = 2.7 Hz, 1H), 7.47 (d, *J* = 9.1 Hz, 1H), 7.25 - 7.21 (m, 1H), 6.94 - 6.85 (m, 3H), 4.35 - 4.25 (m, 2H), 3.76 - 3.50 (m, 4H), 3.50 - 3.38 (m, 2H), 3.28 - 3.11 (m, 2H), 2.11 - 2.00 (m, 2H), 1.79 - 1.62 (m, 4H), 1.60 (s, 3H), 1.57 - 1.49 (m, 2H), 1.10 (s, 9H). LC-MS: m/z 480 [MH^+].

6-({9-[(2-Ethyl-2-propyl-1,3-benzodioxol-4-yl)methyl]-3,9-diazaspiro[5.5]undec-3-yl}carbonyl)pyridin-3-amine trifluoroacetate (29). ^1H NMR (400 MHz, CD_3OD) δ 8.00 (s, 1H), 7.63 - 7.53 (m, 1H), 7.43 - 7.33 (m, 1H), 6.91 - 6.83 (m, 3H), 4.30 (s, 2H), 3.74 - 3.56 (m, 4H), 3.47 - 3.39 (m, 2H), 3.25 - 3.14 (m, 2H), 2.08 - 1.89 (m, 6H), 1.80 - 1.63 (m, 4H), 1.57 - 1.41 (m, 4H), 1.02 - 0.92 (m, 6H). LC-MS: m/z 480 [MH^+].

2-Methyl-2-(2-methylphenoxy)propanoic acid (33). *o*-Cresol (**32**) (21.6 g, 0.2 mmol) and ethyl-2-bromoisobutyrate (60 mL, 0.4 mmol) were dissolved in dry DMF (50 mL). Potassium carbonate (55 g, 0.4 mol) was added and the mixture stirred under argon at 90°C over night. After extraction from heptane/water (500 mL/250 mL) the organic phase was washed with 2M sodium hydroxide (100 mL), water (3x100 mL) and evaporated to give the intermediate ester as an oil (29.8 g). The oil was stirred in sodium hydroxide (24 g, 0.6 mol) dissolved in water (100 mL) at 70°C until a clear solution was obtained (2 h). After acidification with 35% hydrochloric acid the precipitated oil was taken up in

heptane (500 mL), washed with water and evaporated to give crude product (24 g). Recrystallization from heptane (50 mL) yielded pure product as a white solid (19.8 g, 51%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.71 (bs, 1H), 7.18 (d, *J* = 7.4 Hz, 1H), 7.11 (t, *J* = 7.7 Hz, 1H), 6.96 (t, *J* = 7.4 Hz, 1H), 6.83 (d, *J* = 7.3 Hz, 1H), 2.26 (s, 3H), 1.64 (s, 6H). GC-MS m/z 194 [M^+].

2-Methyl-2-(2-methylphenoxy)propanoyl chloride (34). 2-Methyl-2-(2-methylphenoxy)propanoic acid (9.7 g, 50 mmol) was dissolved in thionylchloride (25 mL) and stirred at 50°C 1h. After evaporation the crude product was obtained as an orange oil (10.7 g). The crude product was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (d, *J* = 7.4 Hz, 1H), 7.10 (t, *J* = 7.7 Hz, 1H), 6.96 (t, *J* = 7.4 Hz, 1H), 6.73 (d, *J* = 7.3 Hz, 1H), 2.26 (s, 3H), 1.68 (s, 6H). GC-MS m/z 370 [M^+]anhydride.

2,2,7-Trimethyl-1-benzofuran-3(2H)-one (35). 2-Methyl-2-(2-methylphenoxy)propanoyl chloride (10.7 g, 50 mmol) was dissolved in toluene (250 mL), alumina chloride (6.7 g, 50 mmol) added and the mixture stirred at ambient temp. 1h. After extraction with 1M sodium hydroxide (250 mL), water (3x250 mL) and evaporation of the organic phase the crude product was obtained as an oil. Purification on silica (heptane/ethylacetate 19/1) yielded pure compound as a solid (3.7 g, 42%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, *J* = 7.7 Hz, 1H), 7.43 (d, *J* = 7.3 Hz, 1H), 6.98 (t, *J* = 7.4 Hz, 1H), 2.32 (s, 3H), 1.48 (s, 6H). GC-MS m/z 176 [M^+].

2,2,7-Trimethylspiro[1-benzofuran-3,2'-[1,3]dithiolane] (36). 2,2,7-Trimethyl-1-benzofuran-3(2H)-one (2.0 g, 11.4 mmol) was dissolved in chloroform (10 mL). Ethandithiol (2.8 mL, 34 mmol) and boron trifluoride etherate (2.7 mL, 22 mmol) were

added and the mixture refluxed 3h. The mixture was extracted with 3M sodium hydroxide (40 mL), the organic phase washed with water, dried and evaporated to give the pure compound as a solid (3.05 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (d, J = 7.4 Hz, 1H), 6.99 (d, J = 7.8 Hz, 1H), 6.85 (t, J = 7.4 Hz, 1H), 3.52 - 3.42 (m, 2H), 3.35 - 3.26 (m, 2H), 2.20 (s, 3H), 1.60 (s, 6H). LC-MS m/z 253 [MH^+].

5-Bromo-3,3-difluoro-2,2,7-trimethyl-2,3-dihydro-1-benzofuran (37). 1,3-Dibromo-5,5-dimethylhydantoin (2.86 g, 10 mmol) was stirred under argon at 0°C in dry dichloromethane (25 mL) in a plastic bottle. 70% HF/pyridine-complex (1.8 mL, 70 mmol HF) was added with a plastic syringe followed by 2,2,7-trimethylspiro[1-benzofuran-3,2'-[1,3]dithiolane] (874 mg, 3.5 mmol) dissolved in dry dichloromethane (20 mL). After stirring for 1h the mixture was diluted with dichloromethane (50 mL) and poured onto basic alumina oxide (46 g). The solid was filtered and washed with dichloromethane. Combined filtrate and washing was evaporated to give pure compound as an orange oil (950 mg, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 - 7.41 (m, 1H), 7.36 - 7.33 (m, 1H), 2.20 (s, 3H), 1.50 (t, J_{HF} = 2.1 Hz, 6H). ^{19}F NMR (282.199 MHz, $CDCl_3$) δ -99.19 (2F, s). GC-MS m/z 276/278 1:1 [M^+].

5-Bromo-7-(bromomethyl)-3,3-difluoro-2,2-dimethyl-2,3-dihydro-1-benzofuran (38). 5-Bromo-3,3-difluoro-2,2,7-trimethyl-2,3-dihydro-1-benzofuran (2.0 g, 7 mmol) was dissolved in carbon tetrachloride (20 mL) and NBS (2.6 g, 14 mmol) and benzoylperoxide (40 mg) added. The mixture was stirred at 67°C over night. Precipitated succinimid was filtered off and the filtrate evaporated. Purification on silica (heptane /ethylacetate in gradient) yielded pure compound as a colourless oil (815 mg, 33%). ^{1}H

NMR (300 MHz, $CDCl_3$) δ 7.55 (t, J = 1.2 Hz, 2H), 4.40 (s, 2H), 1.53 (t, J_{HF} = 2.1 Hz, 6H). ^{19}F NMR (282.199 MHz, CDCl3) δ -99.60 (s, 2F). GC-MS m/z 356 [M^+].

***tert*-Butyl 9-[(5-bromo-3,3-difluoro-2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl)methyl]-3,9-diazaspiro[5.5]undecane-3-carboxylate (39).** *tert*-Butyl 3,9-diazaspiro[5.5]undecane-3-carboxylate (484 mg, 1.9 mmol) was dissolved in THF (10 mL) and diisopropylethylamine (1 mL, 6 mmol) was added followed by 5-Bromo-7-(bromomethyl)-3,3-difluoro-2,2-dimethyl-2,3-dihydro-1-benzofuran dissolved in THF (10 mL). The resulting solution was stirred at room temperature over night and a white precipitate was formed. The mixture was diluted with diethylether (100 mL) and was washed with water. The organic layer was dried over Na_2SO_4 and evaporated to afford tert-butyl 9-[(5-bromo-3,3-difluoro-2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl)methyl]-3,9-diazaspiro[5.5]undecane-3-carboxylate as an oil. The crude product was used without further purification. LCMS m/z 529/531 1:1 [MH^+].

***tert*-Butyl 9-[(3,3-difluoro-2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl)methyl]-3,9-diazaspiro[5.5]undecane-3-carboxylate (40).** *tert*-Butyl 9-[(5-bromo-3,3-difluoro-2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl)methyl]-3,9-diazaspiro[5.5]undecane-3-carboxylate (700 mg, 1.33 mmol) was dissolved in MeOH (28 mL) to form a 50 mM solution. The solution was passed through a Thales H-cube using a 10% Pd/C cartridge at room temperature, 1 bar pressure and flow of 1 mL/min. The processed solution was evaporated to afford tert-butyl 9-[(3,3-difluoro-2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl)methyl]-3,9-diazaspiro[5.5]undecane-3-carboxylate as a gum. The crude product was used without further purification. GC-MS m/z 350 (-Boc group).

3-[(3,3-Difluoro-2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl)methyl]-3,9-diazaspiro[5.5]undecane (41). *tert*-Butyl 9-[(3,3-difluoro-2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl)methyl]-3,9-diazaspiro[5.5]undecane-3-carboxylate (1.1 g, 2.44 mmol) was dissolved in dichloromethane (10 mL) and water (0.3 mL). TFA (10 mL) was added. The mixture was stirred at room temperature for 1 hr, the solvents evaporated and the residue made neutral by ion chromatography (SCX column, eluent NH_4OH in MeOH) to afford 3-[(3,3-difluoro-2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl)methyl]-3,9-diazaspiro[5.5]undecane as an oil (414 mg). The crude material (containing di-F hydrolysed product (7-(3,9-diazaspiro[5.5]undec-3-ylmethyl)-2,2-dimethyl-1-benzofuran-3(2H)-one)) was used without further purification.

6-({9-[(3,3-Difluoro-2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl)methyl]-3,9-diazaspiro[5.5]undec-3-yl}carbonyl)pyridin-3-amine trifluoroacetate (30). The compound was prepared according to the final step of Route A (amide coupling). ^{1}H NMR (300 MHz, CD_3OD) δ 7.93 (d, *J* = 2.6 Hz, 1H), 7.47 (d, *J* = 8.5 Hz, 1H), 7.45 - 7.39 (m, 1H), 7.32 (d, *J* = 8.9 Hz, 1H), 7.10 - 7.01 (m, 2H), 3.71 - 3.63 (m, 2H), 3.56 (s, 2H), 3.53 - 3.46 (m, 2H), 2.55 - 2.45 (m, 4H), 1.64 - 1.51 (m, 6H), 1.49 - 1.44 (m, 8H). LC-MS: m/z 472 [MH^+] main signal 471 [M+].

Table S1. Structure and experimental data of the focused set of CCR8 antagonists.[a]

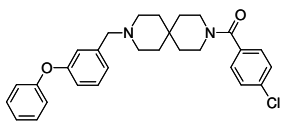
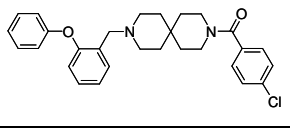
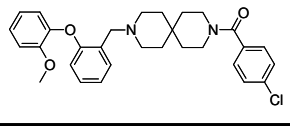
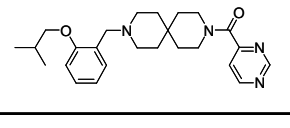
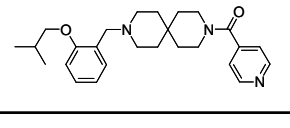
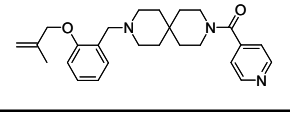
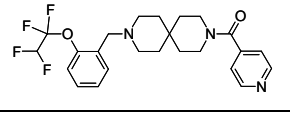
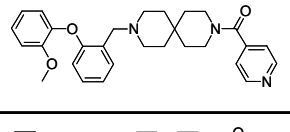
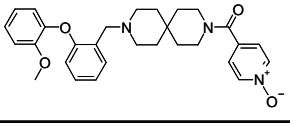
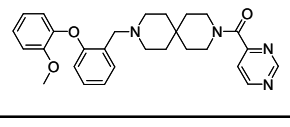
No.	Structure	CCR8	hERGe	hERGb	LogD	T623S	S624A	S624T	Y652A	Y652F	F656M	F656T	F656W
1		7.72	6.22	7.09	3.50	0.60	0.77	0.10	0.42	-0.73	0.42	0.46	0.39
2		7.09	7.08	8.19	3.50	0.91	0.93	0.48	0.53	-0.14	0.78	1.32	-0.08
3		7.46	7.06	8.6	3.40	0.81	0.42	0.08	0.71	-0.20	0.11	0.81	-0.30
4		6.85	5.31	4.72	-0.11	0.22	0.51	0.12	0.70	-0.42	0.21	1.65	-0.42
5		6.97	5.72	4.82	1.80	0.22	0.47	-0.10	1.04	-0.64	0.39	1.82	-0.87
6		7.15	5.63	5.96	1.60	0.08	0.27	0.20	0.74	-1.07	0.23	2.26	-0.60
7		6.28	5.29	4.77	2.80	0.06	0.11	-0.08	1.00	-0.32	0.48	1.42	-0.54
8		7.94	7.34	7.52	1.80								
9		6.94	6.66	6.68	0.50								
10		7.83	7.15	6.92	1.20	0.94	0.58	1.20	1.79	-0.16	0.18	1.49	-0.03

Table S1. Continued 1.

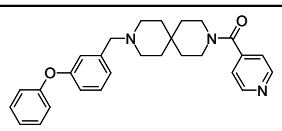
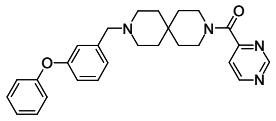
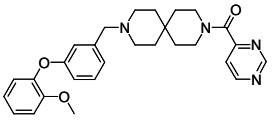
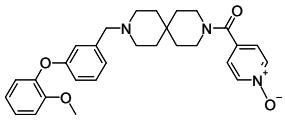
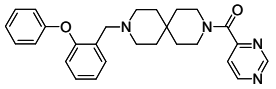
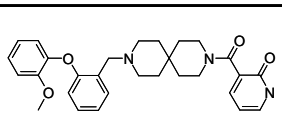
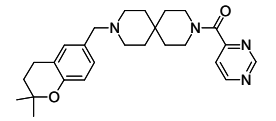
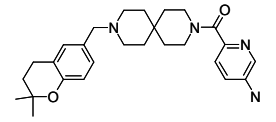
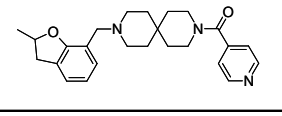
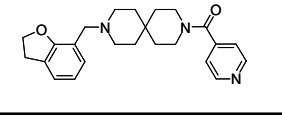
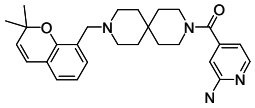
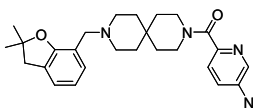
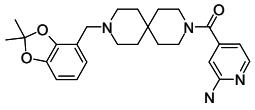
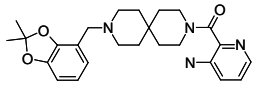
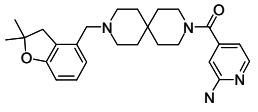
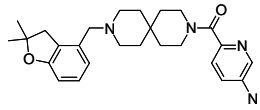
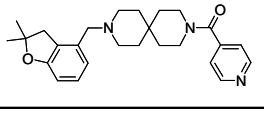
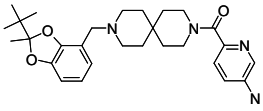
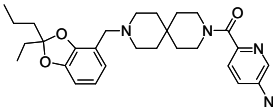
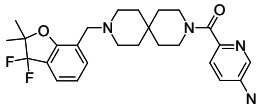
11		7.76	6.35	6.44	2.70								
12		7.56	5.71	5.85	2.40	-0.03	0.56	-0.25	0.67	-0.01	-0.21	0.22	-0.57
13		8.31	5.72		1.70	0.44	0.67	-0.30	0.98	-0.29	-0.20	0.58	-0.15
14		7.49	4.59	5.07	0.90	0.39	0.29	-0.28	1.61	-0.35	-0.20	0.87	-0.39
15		6.85	6.64		2.00	1.16	0.72	0.53	0.79	-0.48	0.21	1.23	-0.16
16		7.26	4.85	5.7	0.90	0.51	0.54	0.24	1.19	0.35	0.22	1.92	-0.18
17		5.74	4.95		0.90	0.39	0.74	-0.39	0.82	0.11	-0.30	0.46	-0.14
18		7.02	4.71	4.49	1.10	0.24	1.09	-0.28	0.83	0.47	-0.66	-0.09	-0.35
19		6.89	4.68	5.09	0.50	0.24	0.61	-0.12	1.63	-0.21	1.04	1.41	-0.58
20		5.83		5.11	0.30								

Table S1. Continued 2.

21		8.25	4.42		1.50	0.20	0.48	-0.62	0.93	0.04	0.37	1.32	-0.75
22		8.58	4.36	4.43	1.00	0.22	0.82	-0.05	0.83	0.35	-0.31	0.51	-0.99
23		8.18	4.78	6.12	1.20	-0.06	0.59	-0.33	1.22	-0.19	0.62	1.37	-0.39
24		7.65	5.38	4.49	1.40	0.41	1.32	0.43	1.81	0.64	-0.26	0.77	-0.32
25		7.65	4.54	4.77	1.40	0.01	0.55	-0.58	0.89	-0.37	0.14	1.32	-0.61
26		8.09	4.78	4.89	1.30	0.49	0.79	0.31	1.23	0.68	-0.22	0.20	-0.66
27		7.51	5.15	4.52	1.60								
28		8.55	4.95	4.51	2.60	0.11	0.70	0.00	0.39	-0.05	-0.73	0.05	-0.58
29		8.62	5.46	5.4	2.50	0.42	0.90	0.17	0.70	0.12	-0.54	0.10	-0.46
30		8.58	4.51	4.32	2.30	0.01	0.36	-0.62	0.87	0.02	-0.49	-0.17	-1.07

[a]CCR8, potency at CCR8 (pIC_{50}^{CCR8}); hERGe and hERGb, potencies (pIC_{50}) at hERG in electrophysiological and binding experiments, respectively; LogD, measured compound lipophilicity; T623S, S624A, S624T, Y652A, Y652F, F656M, F656T and F656W, differences between potencies of the compounds at wild-type hERG and at the

corresponding hERG mutant, ($pIC_{50}^{wt\text{-}hERG} - pIC_{50}^{mutant}$), in electrophysiological experiments.

Table S2. CCR8-ligand H-bond interaction analysis of the focused set.[a]

No.	LLE	$Y^{1.39}$	$Q^{2.60}$	$Q^{45.49}$	$N^{5.39}$	$N^{5.43}$
1	4.22	-	-	-	-	-
2	3.59	-	-	wA	-	-
3	4.06	-	-	wA	-	-
4	6.96	-	-	A	A	A
5	6.17	-	-	A	-	A
6	5.55	-	-	A	-	A
7	3.44	-	-	A	-	-
8	6.14	-	-	A+wA	-	A
9	6.44	-	-	A+wA	-	A
10	6.63	-	-	A+wA	A	A
11	5.06	-	-	wA	-	A
12	5.16	-	-	wA	A	-
13	6.61	A	-	wA	A	A
14	6.59	A	-	wA	-	A
15	4.85	-	-	wA	A	-
16	6.36	-	-	A+wA	A	D
17	4.84	-	-	A	A	A
18	5.92	-	-	A	A	A
19	6.39	-	-	A	-	A
20	5.53	-	-	A	-	A
21	6.75	-	-	A	-	D
22	7.58	-	-	A	A	D
23	6.98	-	A	A	-	D
24	6.25	-	A	A	-	-
25	6.25	-	A	-	-	D
26	6.79	-	A	-	A	D
27	5.91	-	A	-	-	A
28	5.95	-	A	A	A	D
29	5.92	-	A	A	A	D
30	5.28	-	A	A	A	D

[a]The ligand acts as a H-bond acceptor (A) or a H-bond donor (D) to the side chain of the specified CCR8 residue. A weak H-bond accepted by a phenoxy oxygen is indicated with the note “wA”.

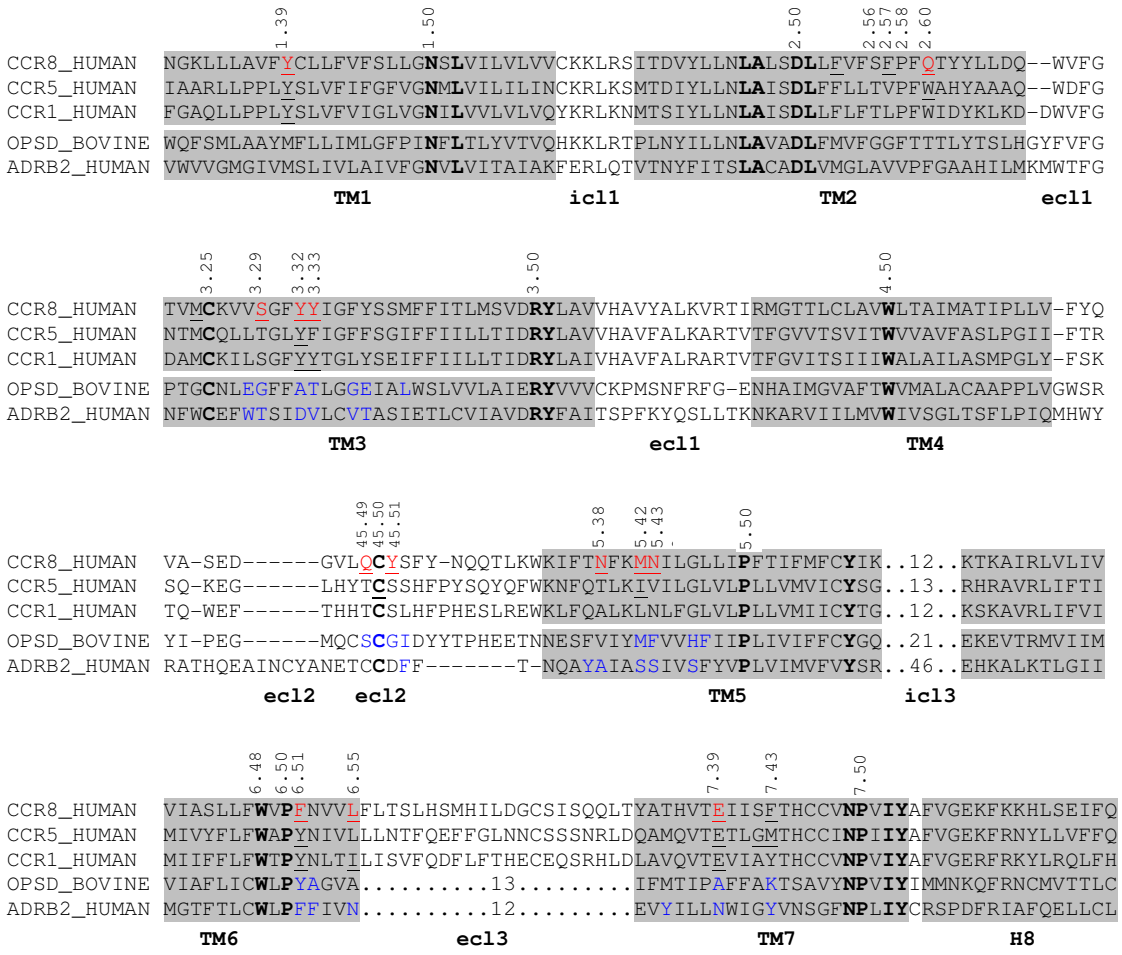


Figure S1. Sequence alignment of chemokine receptors 8 (CCR8), 5 (CCR5), and 1 (CCR1), bovine rhodopsin (OPSD) and the beta 2 adrenergic receptor (ADRB2). Transmembrane domains (TM1 to TM7) and helix 8 (H8) are boxed in grey. Ecl1-3 and icl1-3 indicate the positions of extracellular and intracellular loops. Residues colored in red are used for defining receptor-ligand interaction fingerprints (IFPs). Residues colored in blue are in close contact to the retinal and carazolol in the bovine rhodopsin (Palczewski et al. 2000) and beta 2 adrenergic receptor (Cherezov et al. 2007) crystal structures, respectively. Residues in bold are conserved in all four receptors. The residues referred to in the text of the current paper are underlined. Residue numbers in TMs are

according to Ballesteros-Weinstein (Ballesteros and Weinstein 1995), and residue numbers in ecl2 are according to (de Graaf et al., 2007). Numbers inserted in the icl3 loop describe the number of residues omitted in this study.