Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 16 (2006) 200–203
Bioorganic & Medicinal Chemistry Letters

# Identification and initial evaluation of 4-*N*-aryl-[1,4]diazepane ureas as potent CXCR3 antagonists

Andrew G. Cole,[a,*] Ilana L. Stroke,[a] Marc-Raleigh Brescia,[a] Srilatha Simhadri,[a] Joan J. Zhang,[a] Zahid Hussain,[a] Michael Snider,[b] Christopher Haskell,[b] Sofia Ribeiro,[b] Kenneth C. Appell,[a] Ian Henderson[a] and Maria L. Webb[a]

[a]*Pharmacopeia Drug Discovery, Inc., PO Box 5350, Princeton, NJ 08543, USA*
[b]*Berlex Biosciences, 2600 Hilltop Drive, PO Box 4099, Richmond, CA 94804, USA*

Received 15 August 2005; revised 2 September 2005; accepted 7 September 2005
Available online 5 October 2005

**Abstract**—The identification and evaluation of aryl-[1,4]diazepane ureas as functional antagonists of the chemokine receptor CXCR3 are described. Specific examples exhibit $IC_{50}$ values of ~60 nM in a calcium mobilization functional assay, and dose-dependently inhibit CXCR3 functional response to CXCL11 (interferon-inducible T-cell α chemoattractant/I-TAC) as measured by T-cell chemotaxis, with a potency of approximately 100 nM.

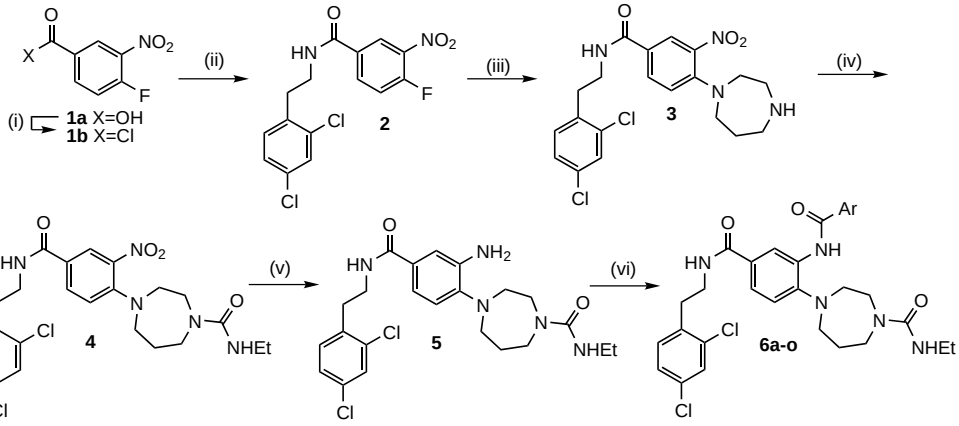

Chemokines are a class of chemotactic cytokines, between 70 and 90 amino acids in length, that play an important role in inflammatory and immune responses. They are subdivided into different classes based on the structural separation of conserved cysteine residues in the chemokine sequence. The CXC subtype of chemokines displays a single amino acid residue between the first two conserved cysteines.[1,2] CXCR3 is a chemokine receptor belonging to the superfamily of seven transmembrane spanning G-protein-coupled receptors (GPCRs) and is highly expressed in activated T cells. Its natural chemokine ligands, CXCL9 (monokine induced by interferon-γ/Mig), CXCL10 (interferon-inducible protein 10/IP-10), and CXCL11 (interferon-inducible T-cell α-chemoattractant/I-TAC),[1,2] are thought to play a key role in directing activated T cells to sites of inflammation.[3] Consequently, inhibition of CXCR3 activation provides potential therapeutic uses for the treatment of a number of inflammatory disorders including inflammatory bowel disease, multiple sclerosis, rheumatoid arthritis, and diabetes.[4] As such, there is significant interest in the identification of small-molecule antagonists of CXCR3.[5]

High-throughput screening of an extensive series of Encoded Combinatorial Libraries on Polymeric Support (ECLiPS™)[6] libraries (90 Libraries, >4 million compounds) employing a FLIPR[7]-based calcium mobilization assay resulted in the identification of 4-aryl-[1,4]diazepane ethyl ureas (**6**) as functional antagonists of CXCR3. Distinct structural features were observed in the screening hits, particularly the [1,4]diazepane ring system and urea functionality, and further investigation was initiated to define the SAR of this class of compounds. Initial benzamide analogs **6** were prepared in six steps as shown in Scheme 1. Thus, 4-fluoro-3-nitro benzoic acid (**1a**) was converted to acid chloride **1b** and reacted with a primary amine to provide amide **2**. N-Arylation of homopiperazine with **2** and subsequent urea formation with ethyl isocyanate was followed by nitro-reduction to the aniline **5** using sodium hydrosulfite[8] under mildly basic conditions. Final functionalization of **5** with a range of acid chlorides provided the target compounds **6** in good overall yield. A similar synthetic approach was used to generate carboxylic acid **11** (Scheme 2) to allow direct modification of the carboxamide substituent on the central aromatic ring via EDC-mediated amide formation.

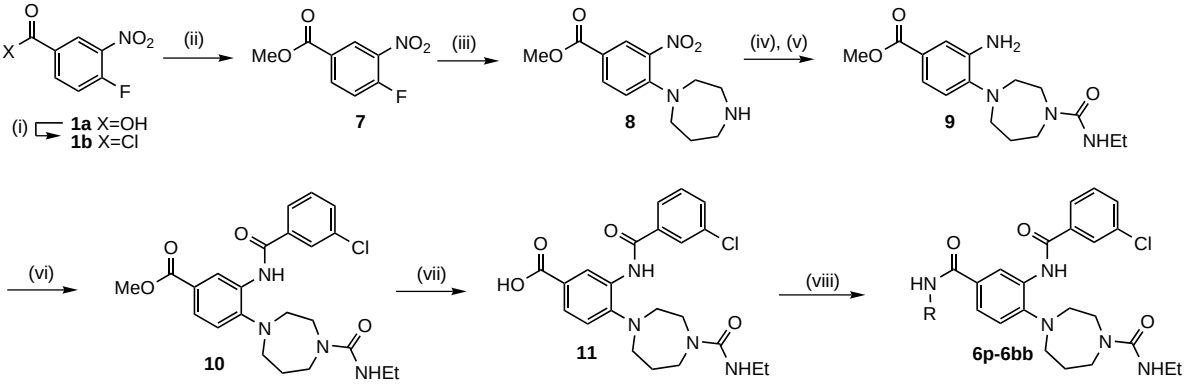
A range of urea and carbamate analogs were also efficiently constructed through utilization of intermediates **3** and **8** to investigate alternative functionalizations of the diazepane ring.

*Keywords*: CXCR3; Chemokine; GPCR; Antagonist; Diazepane; Urea.
* Corresponding author. Tel.: +1 609 452 3653; fax: +1 609 655 4187; e-mail: acole@pcop.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.09.020



**Scheme 1.** Reagents and conditions: (i) $(COCl)_2$, $CH_2Cl_2$, cat. DMF, 0–25 °C; (ii) 2,4-dichlorophenethylamine, $CH_2Cl_2$, 0 °C, 98% from **1a**; (iii) homopiperazine (3.0 equiv), DMF, 0 °C; (iv) EtNCO, $CH_2Cl_2$, 25 °C, 80% from **2**; (v) $Na_2S_2O_4$, $NaHCO_3$, dioxane/MeOH, $H_2O$, 0–25 °C, 68%; (vi) ArCOCl, $Et_3N$, cat. DMAP, $CH_2Cl_2$, 25 °C, 57–86%.



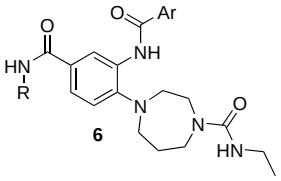
**Scheme 2.** Reagents and conditions: (i) $(COCl)_2$, $CH_2Cl_2$, cat. DMF, 0–25 °C; (ii) MeOH, 0 °C, 99% from **1a**; (iii) homopiperazine (3.0 equiv), DMF, 0 °C; (iv) EtNCO, $CH_2Cl_2$, 25 °C, 95% from **7**; (v) $Na_2S_2O_4$, $NaHCO_3$, dioxane/MeOH, $H_2O$, 0–25 °C, 78%; (vi) 3-ClPhCOCl, $Et_3N$, cat. DMAP, $CH_2Cl_2$, 25 °C, 54%; (vii) LiOH, $H_2O$/dioxane, 85%; (viii) EDC, HOBt, $CH_2Cl_2$, R-$NH_2$, 61–89%.

Functional antagonism was measured by monitoring the reduction in CXCL11-stimulated calcium release for a cell line (HEK293) over-expressing recombinant human CXCR3 and the chimeric G protein Gqi5,[9] following stimulation with 40 nM CXCL11 in a FLIPR-based calcium mobilization assay[10] (no agonist activity was observed for the compounds described). As a specificity control, antagonists were tested at 25 μM for effect on activation of endogenous muscarinic receptors in the same cell line by carbachol. Inhibition was found to be specific for CXCR3. Furthermore, no cytotoxicity was observed in CXCR3 HEK293 cells at 100 μM compound concentration using a WST-1 readout (Roche) after a 24 h incubation.[11]

An investigation of the secondary carboxamide substituent (R) and the benzamide substituent (Ar) was initially conducted to probe the steric and electronic requirements at these two positions for CXCR3 activity. A strong preference for phenethyl-based functionality was observed for the secondary amide, with 2,4-dichloro substitution (**6a–o**) being optimal based on the analogs synthesized (Table 1). Phenethyl-based systems incorporating mono halo substitution (**6p–u**) also maintained reasonable activity superior to the unsubstituted phenethyl derivative (**6v**). Incorporation of the racemic *trans*-cyclopropylphenethyl derivative (**6w**) also displayed a moderate increase in potency in comparison to the parent phenethyl system. The unsubstituted benzylic analog (**6y**) resulted in only moderate activity. However, as observed for the phenethyl-based analogs, a ~5-fold increase in potency was realized on inclusion of a 4-chloro substituent (**6z**). Small aliphatic components such as isopropyl (**6aa**) and cyclopropylmethyl (**6bb**) resulted in a significant reduction in activity, displaying $IC_{50}$ values in the micromolar range. Compounds involving removal of the amide carbonyl acceptor (not shown) displayed no activity versus CXCR3.

Evaluation of the benzamide substituent (Ar) showed good activity for the unsubstituted system (**6a**) and electron-deficient systems substituted in the 3-position (**6c**, **6e**, and **6g**), achieving potencies of 60–80 nM. The analogous 2-substituted derivatives (**6b** and **6f**) resulted in a ~10-fold reduction in potency. Inclusion of simple heterocycles was tolerated (**6k–o**); however, the 4-pyridyl analog (**6m**) displayed significantly better potency than the corresponding 3-pyridyl derivative (**6l**) (Table 1).

**Table 1.** $IC_{50}$ values for 4-*N*-aryl-[1,4]diazepane ethyl ureas




| Compound | R | Ar | $IC_{50}$ ± SEM[a] (μM) |
|---|---|---|---|
| **6a** | 2,4-Dichlorophenethyl | Ph | 0.07 ± 0.00 |
| **6b** | 2,4-Dichlorophenethyl | 2-Cl Ph | 0.70 ± 0.14 |
| **6c** | 2,4-Dichlorophenethyl | 3-Cl Ph | 0.06 ± 0.01 |
| **6d** | 2,4-Dichlorophenethyl | 3-MeO Ph | 0.17 ± 0.03 |
| **6e** | 2,4-Dichlorophenethyl | 3-CN Ph | 0.08 ± 0.03 |
| **6f** | 2,4-Dichlorophenethyl | 2-F Ph | 0.59 ± 0.11 |
| **6g** | 2,4-Dichlorophenethyl | 3-F Ph | 0.06 ± 0.01 |
| **6h** | 2,4-Dichlorophenethyl | 4-F Ph | 0.20 ± 0.04 |
| **6i** | 2,4-Dichlorophenethyl | 3,4-$F_2$ Ph | 0.29 ± 0.07 |
| **6j** | 2,4-Dichlorophenethyl | 3,5-$F_2$ Ph | 0.24 ± 0.12 |
| **6k** | 2,4-Dichlorophenethyl | 2-Thiophene | 0.13 ± 0.02 |
| **6l** | 2,4-Dichlorophenethyl | 3-Pyridyl | 3.10 ± 0.40 |
| **6m** | 2,4-Dichlorophenethyl | 4-Pyridyl | 0.25 ± 0.05 |
| **6n** | 2,4-Dichlorophenethyl | 3-Thiophene | 0.49 ± 0.01 |
| **6o** | 2,4-Dichlorophenethyl | 2-Furan | 0.64 ± 0.11 |
| **6p** | 2-Chlorophenethyl | 3-Cl Ph | 0.19 ± 0.04 |
| **6q** | 3-Chlorophenethyl | 3-Cl Ph | 0.30 ± 0.08 |
| **6r** | 4-Chlorophenethyl | 3-Cl Ph | 0.12 ± 0.02 |
| **6s** | 2-Fluorophenethyl | 3-Cl Ph | 0.34 ± 0.10 |
| **6t** | 3-Fluorophenethyl | 3-Cl Ph | 0.34 ± 0.10 |
| **6u** | 4-Fluorophenethyl | 3-Cl Ph | 0.32 ± 0.06 |
| **6v** | Phenethyl | 3-Cl Ph | 0.51 ± 0.08 |
| **6w** | Cyclopropylphenethyl[b] | 3-Cl Ph | 0.24 ± 0.06 |
| **6x** | 3,4-Dimethoxyphenethyl | 3-Cl Ph | 0.42 ± 0.06 |
| **6y** | Benzyl | 3-Cl Ph | 1.25 ± 0.41 |
| **6z** | 4-Chlorobenzyl | 3-Cl Ph | 0.25 ± 0.04 |
| **6aa** | $^{i}$Pr | 3-Cl Ph | 2.16 ± 0.44 |
| **6bb** | Cyclopropylmethyl | 3-Cl Ph | 2.15 ± 0.12 |

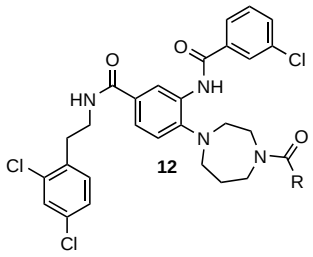
[a] $IC_{50}$ ± standard error mean in response to stimulation with 40 nM CXCL11.
[b] *trans*-Racemic.

Modification of the ethyl urea functionality (Table 2) to generate the *n*-propyl (**12a**), *i*-propyl (**12b**), and *n*-butyl (**12c**) analogs resulted in maintenance of ⩽60 nM activity. Carbon deletion to generate the methyl urea (**12d**) and incorporation of the *N*,*N*-dimethyl tertiary urea (**12e**) resulted in >10-fold reduction in potency. Deviation from the urea functionality to generate the methyl carbamate (**12f**) and ethyl carbamate (**12g**) derivatives resulted in the loss of activity to ⩾10 μM.

Alteration of the central core of **6** to investigate the importance of the disubstituted [1,4]-diazepane ring system was also conducted (not shown). Reduction in ring size to generate the piperazinyl analog, and incorporation of isosteric acyclic variants exhibit no activity against CXCR3. This suggests that the geometry adopted by the [1,4]-diazepane is a preferred conformation of the 1- and 4-substituents for activity.

The activity of **6c** was also measured by monitoring the reduction in CXCL10 (IP-10)-stimulated calcium release

**Table 2.** $IC_{50}$ values for 1-substituted-4-*N*-aryl-[1,4]diazepanes




| Compound | R | $IC_{50}$ ± SEM[a] (μM) |
|---|---|---|
| **6c** | –NHEt | 0.06 ± 0.01 |
| **12a** | –NH$^{n}$Pr | 0.06 ± 0.01 |
| **12b** | –NH$^{i}$Pr | 0.06 ± 0.01 |
| **12c** | –NH$^{n}$Bu | 0.05 ± 0.00 |
| **12d** | –NHMe | 0.80 ± 0.12 |
| **12e** | –$NMe_2$ | 0.89 ± 0.21 |
| **12f** | –OMe | 9.8 ± 1.9 |
| **12g** | –OEt | 13.0 ± 1.7 |

[a] $IC_{50}$ ± standard error mean in response to stimulation with 40 nM CXCL11.

in the same HEK293 cell line.[9] Stimulation with 100 nM CXCL10 in the FLIPR-based calcium mobilization assay resulted in an $IC_{50}$ value of 20 nM for **6c**, demonstrating that antagonism is not limited to receptor activation by CXCL11.

The functional activity of **6c** was further investigated with respect to its ability to inhibit CXCL11 induced T-cell chemotaxis in vitro. Cultured human T cells express both CXCR3 and CCR7, which respond to the ligands CXCL11 and CCL21 (secondary lymphoid-tissue chemokine/SLC), respectively. Compound **6c** successfully inhibited the CXCR3 (CXCL11)-mediated migration, whereas migration through CCR7 (CCL21) was unaffected (Fig. 1).[12] In other experiments **6c** was able to dose-dependently inhibit CXCR3-mediated migration with an $IC_{50}$ of approximately 100 nM.

The specificity of **6c** was also evaluated against a panel of 14 human GPCRs in radioligand binding assays.[13] The GPCRs included adrenergic receptors $\alpha_{2B}$ and $\beta_2$; chemokine receptors CCR1, CCR2B, CCR4, CXCR1 (IL-8A), and CXCR1 (IL-8B); dopamine receptors $D_1$, $D_{2S}$, $D_3$, and $D_{4.2}$; muscarinic receptors M1 and M2; and the serotonin receptor 5-$HT_{1A}$. For each of

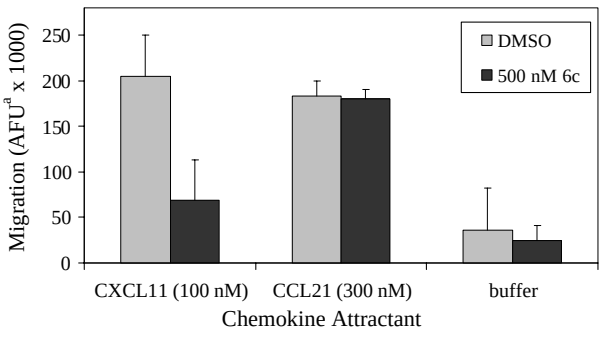



**Figure 1.** Inhibition of CXCL11 induced T-cell chemotaxis by **6c**. [a]Arbitrary fluorescence units.

these GPCRs, less than 50% inhibition was observed at 10 μM.

In conclusion, novel small-molecule functional antagonists of the chemokine receptor CXCR3 have been identified, as determined by inhibition of both calcium mobilization and T-cell chemotaxis on stimulation with CXCL11. In addition, compounds were shown to be non-cytotoxic and display good general selectivity. These initial findings provide a structural template for the further optimization of potency and evaluation of pharmacokinetic properties.

## References and notes


1. Loetscher, M.; Gerber, B.; Loetscher, P.; Jones, S. A.; Piali, L.; Clark-Lewis, I.; Baggioloni, M.; Moser, B. *J. Exp. Med.* **1996**, *184*, 963.
2. Cole, K. E.; Strick, C. A.; Paradis, T. J.; Ogborne, K. T.; Loetscher, M. *J. Exp. Med.* **1998**, *187*, 2009.
3. Murphy, P. M.; Baggiolini, M.; Charo, I. F.; Hebert, C. A.; Horuk, R.; Matsushima, K.; Miller, L. H.; Oppenheim, J. J.; Power, C. A. *Pharmacol. Rev.* **2000**, *52*, 145.
4. (a) Qin, S.; Rottman, J. B.; Myers, P.; Kassam, N.; Weinblatt, M.; Loetscher, M.; Koch, A. E.; Moser, B.; Mackay, C. R. *J. Clin. Invest.* **1998**, *101*, 746; (b) Haskell, C. A.; Ribeiro, S.; Horuk, R. *Curr. Opin. Investig. Drugs* **2002**, *3*, 399; (c) Annunziato, F.; Galli, G.; Cosmi, L.; Romagnani, P.; Manetti, R.; Maggi, E.; Romagnani, S. *Eur. Cytokine Netw.* **1998**, *9*, 12; (d) Yuan, Y. H.; ten Hove, T.; The, F. O.; Slors, J. F.; van Deventer, S. J.; te Velde, A. A. *Inflamm. Bowel Dis.* **2001**, *7*, 281.
5. (a) Ondeyka, J. G.; Herath, K. B.; Jayasuriya, H.; Polishook, J. D.; Bills, G. F.; Dombrowski, A. W.; Mojena, M.; Koch, G.; DiSalvo, J.; DeMartino, J.; Guan, Z.; Nanakorn, W.; Morenberg, C. M.; Balick, M. J.; Stevenson, D. W.; Slattery, M.; Borris, R. P.; Singh, S. B. *Mol. Divers.* **2005**, *9*, 123; (b) Storelli, S.; Verdijk, P.; Verzijl, D.; Timmerman, H.; van de Stolpe, A. C.; Tensen, C. P.; Smit, M. J.; De Esch, I. J. P.; Leurs, R. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2910; (c) Heise, C. E.; Pahuja, A.; Hudson, S. C.; Mistry, M. S.; Putnam, A. L.; Gross, M. M.; Gottlieb, P. A.; Wade, W. S.; Kiankarimi, M.; Schwarz, D.; Crowe, P.; Zlotnik, A.; Alleva, D. G. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 1263.
6. (a) Ohlmeyer, M. H. J.; Swanson, R. N.; Dillard, L.; Reader, J. C.; Asouline, G.; Kobayishi, R.; Wigler, M.; Still, W. C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10922; (b) Nestler, H. P.; Bartlett, P. A.; Still, W. C. *J. Org. Chem.* **1994**, *59*, 4723.
7. Fluorometric Imaging Plate Reader, Molecular Devices.
8. Jones, T. R.; Smithers, M. J.; Taylor, M. A.; Jackman, A. L.; Calvert, A. H.; Harland, S. J.; Harrap, K. R. *J. Med. Chem.* **1986**, *29*, 468.
9. A CXCR3 cDNA clone (sequence as listed in GenBank Accession No. BD195161) and the chimeric G protein Gqi5, cotransfected into HEK293 cells, were used to construct a stable cell line.
10. HEK293/CXCR3 Gqi5 cells were seeded at 10,000 cells (25 μL) per well in poly (D-lysine)-treated 384-well plates (Costar, black clear-bottomed cell culture-treated) 1–2 days prior to the assay. Culture medium was then removed and replaced with 25 μL of 50% cell culture medium/50% Calcium Plus Dye (Molecular Devices)/ 2.5 mM probenecid. For dye loading, plates were incubated for 30 min at 37 °C/5% $CO_2$, followed by equilibration to room temperature for 30–90 min. Test compounds were diluted in 20 μL Hanks' balanced salt solution (HBSS)/20 mM HEPES, pH 7.5/1% DMSO/ 0.1% BSA/2.5 mM probenecid. 12.5 μL test compound (or as controls, CXCL11 to 40 nM or buffer, also with 1% DMSO) was added in the FLIPR 384 to dye-loaded cells. 12.5 μL CXCL11, in HBSS/20 mM HEPES, pH 7.5/ 0.1% BSA, was then added to the cells/test compound, to 40 nM, and fluorescence measured over 3 min.
11. 20,000 HEK293/CXCR3 Gqi5 cells were seeded in clear 96-well tissue culture-treated plates in 50 μL, in culture medium without DMSO. Fifty microliters of the test compounds (serially diluted in medium/2% DMSO) or Triton X-100/2% DMSO as a control was added, followed by incubation for 24 h at 37 °C/$CO_2$. 10 μL WST-1 reagent (Roche) was added and plates were incubated at 37 °C until color developed. After agitation of the plates for 5 min, absorbance at 450 nm was measured.
12. Human peripheral blood mononuclear cells were cultured in media (RPMI + 10% fetal calf serum) with 10% Human T-Cell Growth Factor, (purified Interleukin-2, Advanced Biotechnologies). Following 7–21 days of culture, T cells were washed and resuspended to $10^7$ cells/mL (HBSS, 20 mM HEPES, pH 7.4, and 0.05% BSA). Multiscreen MIC chemotaxis chambers (3 μm, Millipore) were used adding cells (100 μL, $10^6$ cells/well) to the top chamber with 100 nM CXCL11 or 300 nM CCL21 ± antagonist (130 μL) added to the bottom chamber, followed by incubation (90 min at 37 °C). Following migration, Calcein-AM (14 μL, 25 μg/ mL) was added to lower wells. After a 30 min incubation at 37 °C, fluorescence was measured (Victor, Perkin Elmer; 450 $nm_{ex}$/530 $nm_{em}$).
13. Evaluation against a panel of 14 human GPCRs conducted at MDS Pharma-Services measuring % inhibition at 10 μM.