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Design, synthesis and structure–activity relationships of zwitterionic spirocyclic compounds as potent CCR1 antagonists

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ABSTRACT

A series of zwitterionic spirocyclic compounds were synthesised. In vitro data revealed that these compounds were potent CCR1 antagonists. In particular, **2**, **4**, **11** and **20** inhibited CCR1 mediated chemotaxis of THP-1 cells in a functional assay.

Leukocytes trafficking is an important aspect of immune response against pathogens but, to prevent inappropriate response against healthy host cells, needs to be tightly controlled. The loss of such tight control can be the cause of inflammatory diseases. For instance, diseases such as multiple sclerosis[1,2] rheumatoid arthritis,[3,4] are characterised by deregulated leukocytes recruitment. Chemokines play an important role in various inflammatory diseases by mediating leukocytes recruitment.[5–8] Chemokines bind to their target receptors on the cell surface of leukocytes, transmitting intracellular signals by activating G-proteins, resulting in movement of the leukocytes towards the source of the chemokines and the desired site of action. Chemokine receptors are thus attractive targets in the development of drugs against autoimmune diseases due to their vital role in regulation of leukocytes trafficking. The *C-C* (cystine–cystine) chemokine receptor-1 (CCR1) and its major endogenous ligands MIP-1α (CCL3) and RANTES (CCL5) play an important role in chronic inflammatory diseases such as rheumatoid arthritis and multiple sclerosis. Inhibition of CCR1 is expected to be beneficial for patients who suffer from such inflammatory disorders. Thus, small synthetic CCR1 antagonists could be useful as therapeutic agents. The search for specific and highly potent chemokine receptor antagonists has recently been an active area of research[9–14] and here-in, we report the discovery of zwitterionic spirocyclic compounds as potent CCR1 antagonists.

Recently, we have reported compounds **1d**, **1e** and **1f** (Fig. 1) from a series of conformationally constrained spirocyclic comounds[15] many of which are highly potent CCR1 antagonists. These compounds contain either an *N*-acetyl or a benzamide moiety as part of a substituted phenol group. Inclusion of the *N*-acetyl moiety was important for CCR1 activity but, being pro-anilinic, has the potential to be problematic due to possible formation of toxic aniline metabolites. Pleasingly, replacement of the *N*-acetyl moiety with the benzamide moiety in a substituted phenol also resulted in potent CCR1 compounds (Fig. 1).[15b] However, these compounds are prone to inhibit the hERG channel which could render them unsuitable as potential drugs. Thus, we subsequently focused our efforts on the identification of analogues devoid of hERG activity, and it was postulated that this may be facilitated by the inclusion of an acidic group. Thus a series of spirocyclic zwitterionic compounds were designed, such as **6** and **8** (Scheme 1) which were synthesised as described below.

The *para*-methoxybenzyl (PMB) protecting group in **3**[15b] was removed by treatment with trifluoroacetic acid (TFA) to afford **4** in 85% yield. The carboxylic acid derivative **2**[15b] was coupled separately with (*S*)-methyl pyrrolidine-3-carboxylate and (*R*)-methyl pyrrolidine-3-carboxylate in the presence of CDI in DMF to afford **5** and **7** which were hydrolysed by treatment with aqueous NaOH to give the zwitterionic compounds **6** and **8** respectively, in acceptable yields (Scheme 1).

Compound **3** was coupled with methyl pyrrolidine-3-carboxylate in the presence of CDI, Et_3N in DMF to afford **9** which was hydrolysed to zwitterionic compound **10**. Finally, the *para*-methoxybenzyl group in **10** was removed by treatment with trifluoroacetic acid to afford final compound **11** in 28% isolated yield over three steps (Scheme 2). This compound was a mixture of diastereomers.

Spirocyclic amine **1a** was treated with 3-bromopropan-1-ol in the presence of potassium carbonate in DMF to give **12** in 79% isolated yield. Compound **12** was treated separately with methyl 4-fluoro-2-hydroxybenzoate, methyl 5-chloro-2-hydroxybenzoate and ethyl 4-chloro-5-cyano-2-hydroxybenzoate under Mitsunobu reaction conditions to afford either derivatives **13**, **15** and **17** respectively in high yields. The ester group of **13**, **15** and **17** was hydrolysed by treatment with aqueous NaOH to quantitatively give the corresponding carboxylic acids **14**, **16** and **18** which were

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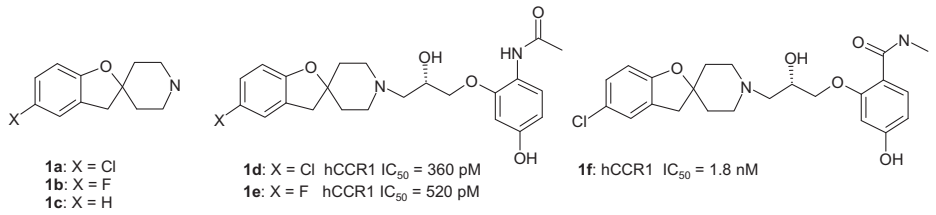
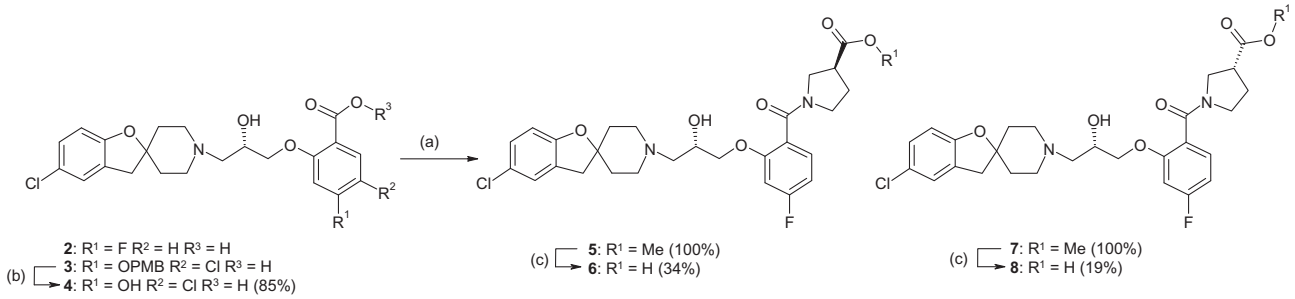


Figure 1. Previously reported CCR1 antagonists.



Scheme 1. Reagents and conditions: (a) (*S*)-methyl pyrrolidine-3-carboxylate/(*R*)-methyl pyrrolidine-3-carboxylate, CDI, DMF, rt, 20 h (100%); (b) TFA, CH_2Cl_2, rt 1 h (85%); (c) Aq. NaOH, EtOH, rt, 3 h (19–34%).

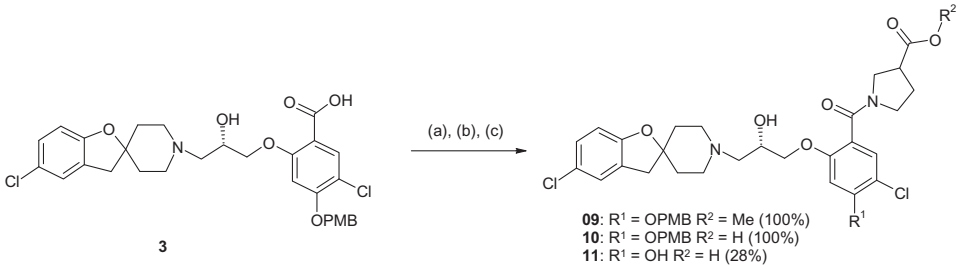
each coupled with (*R*)-methyl pyrrolidine-3-carboxylate in the presence of CDI in DMF to afford **19**, **21** and **23** in high yields, whilst **14** was similarly also coupled with (*S*)-methyl pyrrolidine-3-carboxylate to give **25** in high yield. Finally, the ester group was hydrolysed by treatment with aqueous NaOH to afford zwitterionic compounds **20**, **22**, **24** and **26**, respectively in good yield (Scheme 3).

Spirocyclic amine **1a** was treated with 3-bromo-2,2-dimethylpropan-1-ol in the presence of NaI and Et_3N in DMF to give **28** in low yield. Compound **28** was coupled with methyl 4-fluoro-2-hydroxybenzoate in a Mitsunobu type reaction to afford **29** in good yield. The ester **29** was hydrolysed to give corresponding acid derivative **30** in 70% yield. Compound **30** was coupled with (*R*)-methyl pyrrolidine-3-carboxylate/(*S*)-methyl pyrrolidine-3-carboxylate in the presence of CDI in DMF to give **31** and **33** which were hydrolysed to give zwitterionic compounds **32** and **34** in 30% isolated yield (Scheme 4).

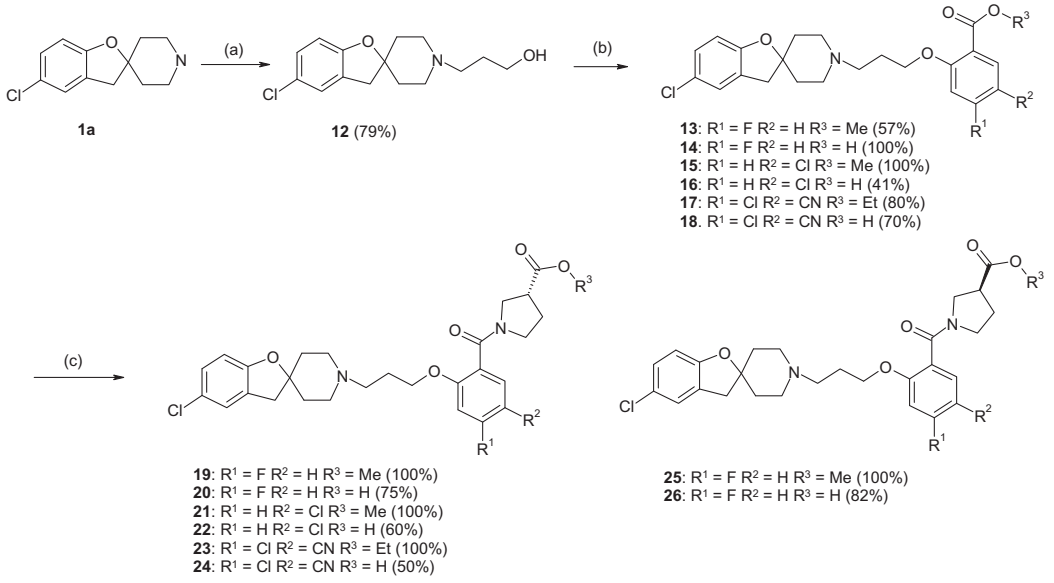
Similarly, compound **14** was coupled with methyl 4,4-dimethylpyrrolidine-3-carboxylate to afford **35** which was hydrolysed to give racemic mixture of zwitterionic compound **36** in 28% overall yield (Scheme 5).

Initially the intermediate carboxylic acid derivatives **2**, **4**, **14** and **16** (also zwitterionic) were evaluated and in vitro data (Table 1) revealed these to be moderately potent CCR1 antagonists, with the most potent being compound **2** (IC_{50} = 20 nM). In addition, **2** and **4** inhibited CCR1 mediated chemotaxis in a functional assay. These substances were also very stable in both human microsomes and hepatocytes as well as rat hepatocytes whilst being inactive against the hERG channel. Compounds **2**, **14** and **16** were found to have high permeabilities as determined in a Caco-2 assay. From initial evaluation, the zwitterion approach appeared to yield compounds with an attractive overall profile, albeit with modest CCR1 potency. Thus, in an attempt to improve CCR1 potency zwitterionic amide derivatives **6**, **8** and **11** were designed and gratifyingly biological evaluation revealed these to be very potent CCR1 antagonists. In particular, **11** inhibited CCR1 mediated chemotaxis in a functional assay (Table 1). As for the earlier zwitterionic compounds, these compounds were also very metabolically stable and free from hERG activity.[16] The permeability of the latter series of compounds was generally very low and, as these compounds were intended for oral administration, it was considered that bioavailability would consequently be limited.

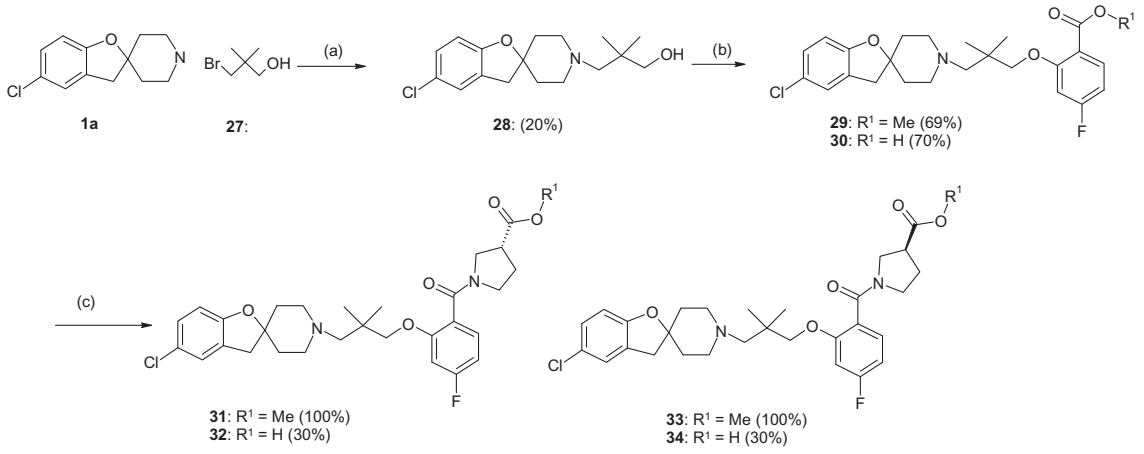
In order to improve permeability **20** and **26** were designed with the hydroxyl group removed from the carbon linker. Pleasingly, the biological data revealed that the hydroxyl group in **20** and **26** was not be essential for CCR1 binding affinity and potent CCR1 antagonist activity was retained in such compounds. Moreover, **20** was the most potent inhibitor of CCR1 mediated chemotaxis of the zwitterion series (Table 1). Although stability in both human microsomes and hepatocytes was similar to previous compounds



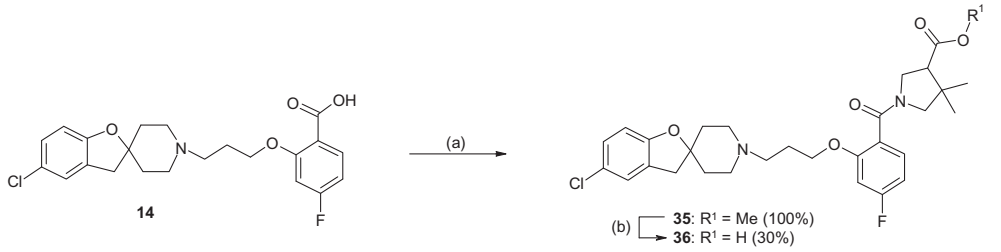
Scheme 2. Reagents and conditions: (a) methyl pyrrolidine-3-carboxylate, CDI, Et_3N, DMF, rt 20 h (100%); (b) aqueous NaOH, EtOH, rt, 3 h (100%); (c) TFA, CH_2Cl_2, rt 1.5 h (28%).



Scheme 3. Reagents and conditions: (a) 3-bromo-propan-1-ol, K_2CO_3, DMF, rt, 2 days (79%); (b) methyl 4-fluoro-2-hydroxybenzoate/methyl 5-chloro-2-hydroxybenzoate/ethyl 4-chloro-5-cyano-2-hydroxybenzoate, Ph_3P, DEAD, THF, rt 20 h (57–100%); (c) (*R*)-methyl pyrrolidine-3-carboxylate/(*S*)-methyl pyrrolidine-3-carboxylate, CDI, DMF, rt, 20 h (100%).



Scheme 4. Reagents and conditions: (a) NaI, Et_3N, DMF, 78 0C 60 h (20%); (b) methyl 4-fluoro-2-hydroxybenzoate, Ph_3P, DEAD, THF, rt 20 h (69%); (c) (*R*)-methyl pyrrolidine-3-carboxylate/(*S*)-methyl pyrrolidine-3-carboxylate, CDI, DMF, rt, 20 h (100%).



Scheme 5. Reagents and conditions: (a) methyl 4,4-dimethylpyrrolidine-3-carboxylate, CDI, DMF, rt, 20 h (100%); (b) aqueous NaOH, EtOH, rt, 3 h (30%).

in this series, regrettably moderate activity against the hERG channel was observed whilst improvement in permeabilities for these compounds was somewhat modest. The removal of hydroxyl group from the carbon linker and incorporation of a *para* chlorine instead of a *meta* fluorine in a substituted phenol as in **22** resulted in a nearly 26-fold less potent CCR1 antagonist compared to **20**. In addition, **22** was a potent inhibitor of hERG channel and the permeability was moderate.

Next, we wanted to investigate whether the substitution of *para* chloro with a nitrile group in a substituted phenol would be tolerated with regard to CCR1 potency. Thus, **24** was designed and synthesised. The biological data revealed that CCR1 binding affinity was lost completely in such compound. In an attempt to investigate the tolerability with regard to CCR1 potency and the effect on pharmacokinetic properties **32** and **34** were designed with gem dimethyl substitution incorporated into the carbon linker.

Table 1
potent CCR1 antagonists[17]

Entry	hCCR1 MIP1α IC_{50} (μM)	hheps	hmics	Caco2	rheps	hERG IC_{50} (μM)	Chemotaxis MIP1α IC_{50} (μM)	PSA (Å^2)	*c*log*P*
2	0.02	<3	<3	9	<3	>25.6	0.25	83	1.67
4	0.022	<3	<10	1.1	<3	61.7	0.093	106	1.53
6	0.0045	<3	<10	0.2	N.a	81.9	N.a	102	0.92
8	0.0032	<3	<10	0.35	<3	>81.9	N.a	102	0.92
11	0.0056	<3	<10	1.8	<3	>100	0.024	125	0.92
14	0.089	<3	<10	22	<3	1.3	N.a	60	2.83
16	0.24	N.a	<10	27	N.a	N.a	N.a	60	3.4
18	>3.2	N.a	<10	2.5	N.a	7.9	N.a	79	2.93
20	0.0014	<3	<10	1.4	4.3	14.1	0.0067	80	2.08
22	0.037	<3	10	2.4	N.a	1.9	N.a	80	2.65
24	1.7	N.a	<10	1.6	N.a	N.a	N.a	98	2.11
26	0.0092	<3	<10	1.2	N.a	10.7	N.a	80	2.08
30	>3.2	N.a	<10	N.a	N.a	N.a	N.a	60	3.63
32	1.6	N.a	<10	N.a	N.a	N.a	N.a	80	2.88
34	2.5	N.a	<10	N.a	N.a	N.a	N.a	80	2.88
36	0.3	N.a	<10	1.2	N.a	N.a	N.a	80	3.12

Data represents average value of at least two experiments.
N.a: not available.
hheps: Human hepatocytes (μl/min/10^6cells).
rheps: Rat hepatocytes (μl/min/10^6cells).
hmic: Shuman microsomes (μl/min/mg).
Caco-2: human colonic adenocarcinoma (cm/s $\times 10^6$).
hERG: human ether-a-go-go-related-gene.
MIP-1α: macrophage inflammatory protein-1-alfa.
PSA: molecular polar surface area.

The in vitro biological evaluation revealed these compounds to have greatly reduced CCR1 binding affinity without the anticipated improvement in permeability. Next, we introduced a gem dimethyl group in the pyrrolidine moiety to investigate the tolerability with regard to CCR1 potency and impact on pharmacokinetic properties of such a compound. Thus, **36** was designed and synthesised. The incorporation of a gem dimethyl group in the pyrrolidine ring yielded less potent CCR1 antagonist and the permeability did not improve.

In conclusion, there was no clear correlation between permeability and *c*log*P* for this class of zwitterionic compounds (Table 1). For example, the *c*log*P* of **2** was lower than, **18**, **20** and **22** but the permeability was good despite the molecular polar surface area (PSA) being the same for these compounds. The *c*log*P* of **14** was comparable to **18** but the permeability of **14** was much higher. There was no correlation between permeability and PSA either. The PSA of **2**, **18**, **20**, **22**, **26** and **36** was similar but only **2** had good permeability. The lipophilicity of **6**, **8** and **11** was same (*c*log*P*, 0.92) but the permeability of **6** and **8** was very low despite **11** had higher PSA. The permeability of **14** (*c*log*P*, 2.83) was remarkably higher than that of **18** (*c*log*P*, 2.93) and **22** (*c*log*P*, 2.65) despite the *c*log*P* of these compounds being comparable. The high permeability of **14** could be attributed to low PSA (60 Å^2) whilst that of **18** and **22** with PSA 79 Å^2 and 80 Å^2, respectively had lower permeability. There was a tendency to exhibit low hERG channel activity with low *c*log*P*. The only exception was observed for compound **18** which exhibited to some extent lower hERG channel activity compared to **14** despite *c*log*P* was comparable for these compounds.

In this study a series of zwitterionic compounds which were very potent CCR1 antagonists in vitro, with excellent metabolic stabilty and devoid of hERG channel activity were designed and synthesised. In addition, this class of compounds inhibited CCL3 mediated chemotaxis of THP-1 cells in a functional assay. In particular, **11** and **20** were very potent CCR1 antagonists with excellent in vitro metabolic stability and these compounds were also the most potent inhibitor of chemotaxis[18] of THP-1 cells in this zwitterionic series. The hERG channel activity of **20** was modest whilst **11** was totally inactive in hERG though the permeabilty was borderline. Thus, further investigations will be required to optimise such parameters.

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16. hERG IC_{50} was determined in human embryonic kidney cells via ion flux electrophysiology, for detail see Ref. 15b.
17. *Human CCR1 Membrane:* HEK293 cells, from ECACC, stably expressing recombinant human CCR1 (HEK-CCR1) were used to prepare cell membranes containing CCR1. The membranes were stored at −70 °C. The concentration of membranes of each batch was adjusted to 10% specific binding of 33 pM [^{125}I] MIP-1α
Human CCR1 binding assay: 100 μl of HEK-CCR1 membranes diluted in assay buffer pH 7.4 (137 mM NaCl (Merck), 5.7 mM glucose (Sigma) 2.7 mM KCl (Sigma), 0.36 mM $NaH_2PO_4 \times H_2O$ (Merck), 10 mM HEPES (Sigma), 0.1% (w/v) Gelatine (Sigma) with the addition of 17500 units/l Bacitracin (Sigma) were added to each well of the 96-well filter plate (0.45 μM opaque Millipore), 12 μl of compound in assay buffer containing 10% DMSO was added to give final compound concentration of $1 \times 10^{-5.5}$–$1 \times 10^{-9.5}$ M. 12 μl Old human recombinant MIP-1α (R & D systems), 10 nM final concentration in assay buffer supplemented with 10% DMSO, was included in certain wells (without compound) as non specific binding control (NSB). 12 μl Assay buffer with 10% DMSO was added to certain wells (without compound) to detect maximal binding (B0). 12 μl [^{125}I] MIP-1α diluted in assay buffer to a final concentration in the wells of 33 pM, was added to all wells. The plates with lid were then incubated for 1.5 h at room temperature. After incubation the wells were emptied by vaccum filtration (MultiScreen Resist Vacuum Manifold System, Millipore) and washed once with 200 μl assay buffer. After the wash, all wells received an addition of 50 μl of scintillation fluid (OptiPhase Supermix, Wallac Oy). Bound [^{125}I] MIP-1α was measured using a wallac Trilux 1450 MicroBeta counter.Calculation of percent displacement and IC_{50}. The following equation was used to calculate percent displacement. Percent displacement = 1−{(cpm test−cpm NSB)/(cpmB0−cpmNSB)} where cpm test = average cpm in wells with membranes and compound and [^{125}I] MIP-1α, NSB = average cpm in the wells with membranes and MIP-1α and [^{125}I] MIP-1α (non specific binding). B0 = average cpm in well with membranes and assay buffer and [^{125}I] MIP-1α (maximum binding). The molar concentration of compound producing 50% displacement (IC_{50}) was derived using the Excel XL fit (version 2.0.9) to fit data to a 4-parameter logistics function.
18. *Culture of THP-1 cells:*
Cells were thawed rapidly at 37 °C from frozen aliquots and resuspended in a 25 cm flask containing 5 ml of RPMI-1640 medium supplemented with Glutamax and 10% heat inactivated fetal calf serum without antibiotics (RPMI+10%HIFCS). At day 3 the medium is discarded and replaced with fresh medium. THP-1 cells are routinely cultured in RPMI-1640 medium supplemented with 10% heat inactivated fetal calf serum and glutamax but without antibiotics. Optimal growth of the cells requires that they are passaged every 3 days and the minimum subculture density is 4×10^5 cells/ml.
Chemotaxis assay:
Cells were removed from the flask and washed by centrifugation in RPMI+10% HIFCS + glutamax. The cells were then resuspended at 2×10^7 cells/ml in fresh medium (RPMI+10% HIFCS + glutamax) to which was added calcein-AM (5 μl of stock solution to 1 ml to give a final concentration of 5×10^{-6} M). After gentle mixing the cells were incubated at 37 °C in a CO_2 incubator for 30 min. The cells were then diluted to 50 ml with medium and washed twice by centrifugation at 400×*g*. Labelled cells were then resuspended at a cell concentration of 1×10^7 cells/ml and incubated with an equal volume of MIP-1α antagonist (10^{-10}–10^{-6} M final concentration) for 30 min at 37 °C in a humidified CO_2 incubator. Chemotaxis was performed using Neuroprobe 96-well chemotaxis plates employing 8 μm filters (cat no.101-8). Thirty μl of chemoattractant supplemented with various concentrations of antagonists or vehicle were added to the lower wells of the plate in triplicate. The filter was then carefully positioned on top and then 25 μl of cells preincubated with the corresponding concentration of antagonist or vehicle were added to the surface of the filter. The plate was then incubated for 2 h at 37 °C in a humidified CO_2 incubator. The cells remaining on the surface were then removed by adsorption and whole plate was centrifused at 2000 rpm for 10 min. The filter was then removed and the cells that had migrated to the lower wells were quantified by the fluorescence of cell associated calcein-AM. Cell migration was then expressed in fluorescence units after subtraction of the reagent blank and values were standardized to% migration by comparing the fluorescence values with that of a known number of labelled cells. The effect of antagonists was calculated as % inhibition when the number of migrated cells was compared with vehicle.