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Overcoming hERG activity in the discovery of a series of 4-azetidinyl-1-aryl-cyclohexanes as CCR2 antagonists

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ABSTRACT

A series of 4-azetidinyl-1-aryl-cyclohexanes as potent CCR2 antagonists with high selectivity over activity for the hERG potassium channel is discovered through divergent SARs of CCR2 and hERG.

Chemokines are a family of chemoattractant cytokines (CC) whose main function is to regulate the trafficking and activation of leukocytes and other cell types under a variety of inflammatory and noninflammatory conditions. CCL2, commonly referred to as monocyte chemotactic protein-1 (MCP-1), is one of the primary molecules controlling the influx of mononuclear leukocytes into sites of inflammation. MCP-1 is a potent chemotactic factor for monocytes and memory T lymphocytes, and stimulates the movement of those cells along a chemotactic gradient following binding to its cell-surface receptor, CC chemokine receptor-2 (CCR2). This ligand/receptor pair is overexpressed in numerous inflammatory conditions wherein excessive monocyte recruitment is observed, such as atherosclerosis and rheumatoid arthritis.[1–5] An antagonist of the binding of MCP-1 to its receptor CCR2 may be an effective treatment for any inflammatory disease in which monocytes, mast cells, or basophils play major roles. Such diseases include, among others, asthma, obesity, insulin resistance, multiple sclerosis, atherosclerosis, inflammatory bowel diseases, anterior uveitis, gingivitis, periodontitis, allergic rhinitis, allergic conjunctivitis, inflammatory acne, psoriasis, and atopic dermatitis.[6–15]

Over the past several years, there has been intensive research interest in small molecule antagonists of the CCR2 receptor resulting in the disclosure of many distinct chemical series.[16–20] As the work described within this paper was being carried out, literature examples of CCR2 antagonists have been reported within a range of 'chemotypes'.[21–33] Of all reported chemical series, many CCR2 antagonists fall into a general class with a 'sandwich' structure consisting of a central basic amine flanked by two hydrophobes. The dominance of this chemotype is consistent with the importance of the interaction between glutamic acid-291 on CCR2 residues and ligands. MK-0812, INCB3344, JNJ lead and BMS lead are representative examples for this chemotype (Fig. 1).

As part of our intensive efforts on CCR2 antagonists, we started to optimize our lead (JNJ lead in Fig. 1) for eliminating the zwitter ion form and improving solubility. Through synthesis and evaluation of a large number of templates containing various heterocyclic substitutions built around a central basic amine, we arrived at

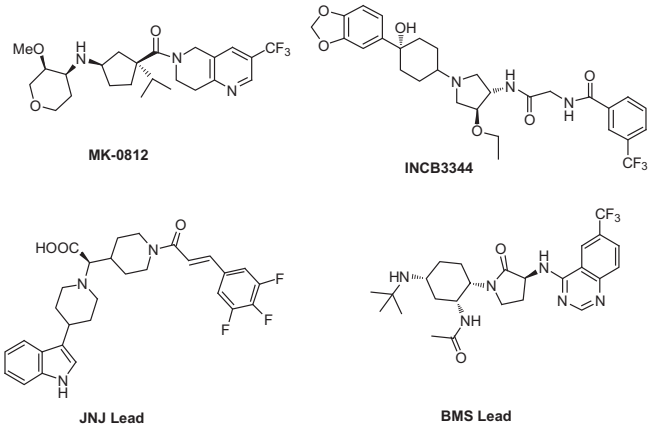

Figure 1. Representative examples of potent CCR2 antagonists.

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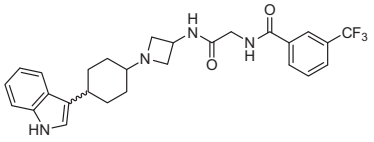
compound **1** as *cis–trans* cyclohexyl mixtures bearing azetidinyl feature with sub μM CCR2 binding affinity. We were particularly interested in this scaffold since it eliminated the zwitter ion and incorporated a soluble bis-amide side chain, which could provide compounds with amendable pharmaceutical properties and synthetic expedience.[34] However, compound **1** was also a potent ligand for the hERG channel displaying IC_{50} of 2.4 μM (Fig. 2). There was no separation between CCR2 and hERG activities. Hence, the challenge was to improve selectivity over hERG ion channel. Herein we describe the SAR and optimization of this azetidine series to the identification of lead compounds with potent CCR2 antagonistic activity and high selectivity versus hERG.

The synthesis of target compounds in this series is straightforward. A general synthetic scheme is shown in Scheme 1. The left-side building blocks **4** were constructed by Suzuki coupling of starting material aryl bromides **2** and vinyl boronic ester **3**[35] in 55–80% yield, from which the final compounds **8** (*cis*-isomer of 1,4-substitutions on the cyclohexanyl ring) and **9** (*trans*-isomer) in ratio of 3:1 to 1:1 and total 65–90% yield were synthesized by simple reductive amination with $NaBH(OAc)_3$ of the azetidine-containing building blocks **7**. Intermediates **7** were prepared by amide coupling (EDCI and HOEt) of glycine acid derivatives **5** and amino azetidine **6** in 78–85% yield. The structure of *cis*-isomer was determined by 2D NMR (COSY and NOSY).

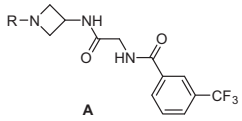
Target compounds were screened by CCR2 binding assays to assess their ability to inhibit MCP-1 binding to human peripheral blood monocytes (MCP-1 assay)[36] and by a CCR2 functional assay to determine their ability to inhibit MCP-1 stimulated chemotaxis in human peripheral blood monocytes (CTX assay).[37] Table 1 shows the initial examples on modifications of the cyclohexanyl ring. The *cis*-isomer **8a** is a potent CCR2 inhibitor with an IC_{50} of 5 nM in the MCP-1 binding assay while the *trans* isomer **9a** is only moderately active in the same assay (IC_{50} of 1.8 μM). Replacement of the *cis* cyclohexanyl group with either the cyclohexenyl or the piperidinyl



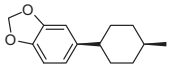
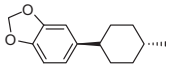
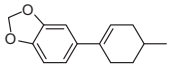
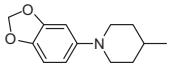
1
hCCR2 binding, IC_{50} < 1 μM
hERG binding, IC_{50} 2.4 μM

Figure 2. Initial hit.

Table 1



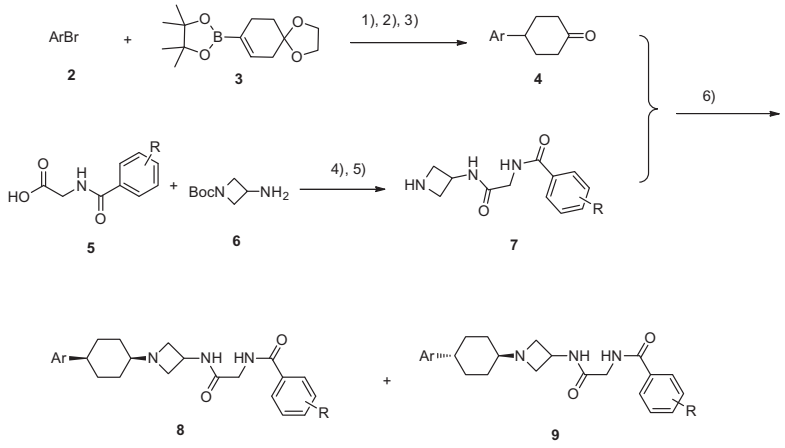
A

ID	R	CCR2 binding[a] IC_{50} (nM)
8a		5[b]
9a		1,800[c]
10		1,000[c]
11		>25,000[c]

[a] MCP-1 receptor binding assay in THP-1 Cells, see Reference 36.
[b] IC_{50} value is reported as the average of five separate determinations.
[c] IC_{50} values are reported from single determination.

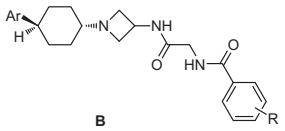
group substantially reduced or completely abolished CCR2 activity, as shown in **10** (IC_{50} of 1.0 μM) and **11** (IC_{50} of >25 μM), which indicated the *cis* geometry of 1-aryl and 4-azetidinyl substitutions on the cyclohexanyl ring is essential for effective ligand/receptor binding.

Having established *cis* 4-azetidinyl-1-aryl cyclohexanyl moiety as the key template which possesses encouraging CCR2 activity, we initiated SAR studies on both phenyl rings on the left and right sides of the molecule. Initial modification on the right side aryl ring indicated that *meta*-CF_3 might be the optimal substituent (Table 2). As illustrated by **8c** and **8d**, either removal of *meta*-CF_3 or moving CF_3 group from *meta* to *para* position resulted in dramatic loss of CCR2 binding affinity. We then turned our attention to the left-end aryl ring. Electron rich phenyl ring attached to 1-position of the cyclohexanyl ring had beneficial effect on the CCR2 activity as evidenced by an approximate three to sevenfold increase of CCR2 binding affinity when a 4-methoxy or 3,4-methylenedioxy group was installed (**8a** and **8e** vs **8b**). The observed high potency of **8a** and **8e** was also translated into excellent levels of activity in the CCR2 chemotaxis assay with IC_{50} of 15 nM and 25 nM, respectively. In contrast, electron withdrawing groups such as carboxyl

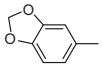
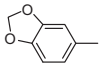
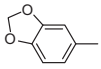
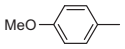
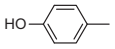
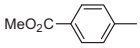
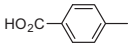
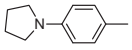
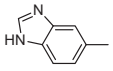


Scheme 1. Reagents: (i) $Pd(Ph_3P)_4$, Na_2CO_3; (ii) H_2, 10% Pd/C, 50 psi; (iii) HCl, acetone; (iv) EDCI, HOBt; (v) H_2SO_4 or TFA; (vi) $NaBH(Ac)_3$, silica gel column separation 5% 7 N NH_3/MeOH in DCM.

Table 2



B

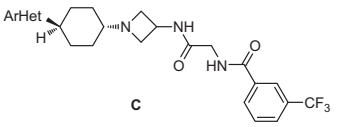
ID	Ar	R	CCR2 binding IC_{50}[a] (nM)	CTX[b] IC_{50} (nM)	hERG binding[c] IC_{50} (μM)	hERG patch[d]% @3 μM
8b		3-CF_3	35	35	4.0	97
8a		3-CF_3	5	15	2.8	95
8c		H	390	nt[e]	2.8	nt
8d		4-CF_3	1200	nt	1.5	nt
8e		3-CF_3	10	25	3.0	99
8f		3-CF_3	21	42	4.1	75
8g		3-CF_3	80	nt	1.3	91
8h		3-CF_3	70	1300	23.0	35
8i		3-CF_3	61	240	7.9	95
8j		3-CF_3	27	21	2.5	88

[a] IC_{50} value are reported as the average of at least two separate determinations.
[b] MCP-1 induced chemotaxis in THP-1 cells (Ref. 37).
[c] hERG 3H-astemizole binding activity on HEK-293 cell (Ref. 38).
[d] The membrane K^+ current IKr in HERG-transfected HEK293 cells using the PATCHXPRESS planar patch-clamp instrument (Ref. 39).
[e] nt: not tested.

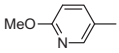
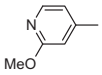
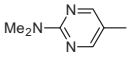
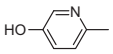
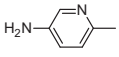
significantly reduced CCR2 binding as indicated with **8g** with IC_{50} of 80 nM. It is well known that structures containing a central basic amine flanked by two hydrophobes are generally good binders for hERG ion channel. It is not surprising that our early analogs showed no selectivity against hERG. Divergent SAR of CCR2 and hERG is required to achieve sufficient cardiovascular safety window. To increase throughput, we used high throughput hERG binding screen[38] followed by hERG patch express[39] assays. This strategy allowed us to evaluate large number of compounds in a rapid fashion. While CCR2 activity could be well maintained in this scaffold when steric bulkiness of the substitutions on the aryl ring was altered as indicated in Table 2, hERG liability seems inseparable. For example, compound **8i** was a potent binder for the hERG channel displaying IC_{50} of 7.9 μM in hERG binding assay. The strong hERG affinity of the compound was further confirmed with 95% of inhibition in hERG patch express assay when screened at 3 μM. To dial out the unwanted hERG signal of this series we initially developed a strategy of increasing the polarity of the molecules. From the initial set of analogs in Table 2, it was apparent that regardless of the polar groups introduced, including mono-substitution (**8f**) and fused ring (**8j**), not much hERG selectivity was achieved except **8h**, a zwitter ion. Unfortunately, beside its weak functional activity in chemotaxis (IC_{50}, 1.3 μM), it did not have amendable pharmaceutical properties for further development.

We then turned our attention to dial out hERG by replacing the left-end phenyl ring with more polar pyridinyl ring. As illustrated in Table 3, while pyridinyl analog **12a** maintained good CCR2 binding affinity (IC_{50}, 45 nM), it still carried strong hERG binding and functional activity. Installation of methoxy group onto the pyridinyl ring (**12b**, **12c** and **12d**) did not show significant improvement on attenuating hERG binding affinity. Replacement of the pyridinyl with more polar pyrazinyl (**12e**) or pyrimidinyl (**12f**) group failed to dial out hERG activity. In contrast to their substituted phenyl analogs in Table 2, pyridines with polar substituents proved to be effective in suppressing hERG in both binding and functional assays. Hydroxy (**12g** and **12h**) or amino pyridines (**12i** and **12j**) reduced hERG binding affinity significantly with maintained CCR2 activity. Encouraged by this finding, we decided to move polar functional groups to 1-poisition of cyclohexanyl ring since it would be located at the C-2 symmetry and hence does not increase complexity for stereochemistry. This strategy was well accomplished as **14a** (Fig. 3) was found to reduce hERG activity dramatically compared with its des-oxy analog **12a** (Table 3).[40] However, it also substantially reduced CCR2 potency. Our next goal was to improve CCR2 activity to achieve good selectivity over hERG. We decided to switch the left side pyridinyl ring back to phenyl ring and examine the impact of the substitution at 1-position of the cyclohexanyl ring on both CCR2 and hERG activity. We prepared a series of 1-substituted analogs according to the synthetic routes in Scheme 2.[39] Addition of aryl lithium generated from compounds **2** with 1,4-cyclohexanedione monoethylene ketal followed by deprotection of the ketal group afforded hydroxyl ketones **13** in about 60% yield for two steps. Ketones **13** were then coupled with amine **7** by reductive amination to give a pair of isomers **14** and **15** in about 3:1 to 1:1 ratio in ~70% yield. The mixtures were then treated with DAST at −78 °C to form a pair of fluorinated azetidines **16** (*cis*-isomer) and **17** (*trans*-isomer) in 80% yield, Reaction of the ketone with *t*-Bu-sulfinamide by $Ti(OEt)_4$ afforded the corresponding sulfinimide **18**, which was then coupled with aryl Grignard reagent generated through metal–halogen exchange followed by

Table 3

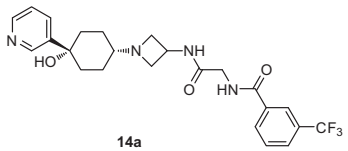


c

ID	HetAr	CCR2 binding IC_{50} [a] (nM)	CTX IC_{50} [a] (nM)	hERG binding IC_{50} (μM)	hERG patch% @ 3 μM
12a		45	52	3.8	92
12b		7	11	2.8	97
12c		32	28	8.2	nt
12d		25	170	6.2	nt
12e		44	nt	12	87
12f		31	nt	9.8	76
12g		13	14	10	36
12h		52	57	23	28
12i		43	35	44	nt
12j		85	nt	37	25

[a] IC_{50} value are reported as the average of at least two separate determinations.

de-protection of the ketal group to give amino ketone **20** in about 40% yield.[41] Compound **20** was then reacted with **7** under reductive amination conditions to afford amino azetidines **21** and **22**.



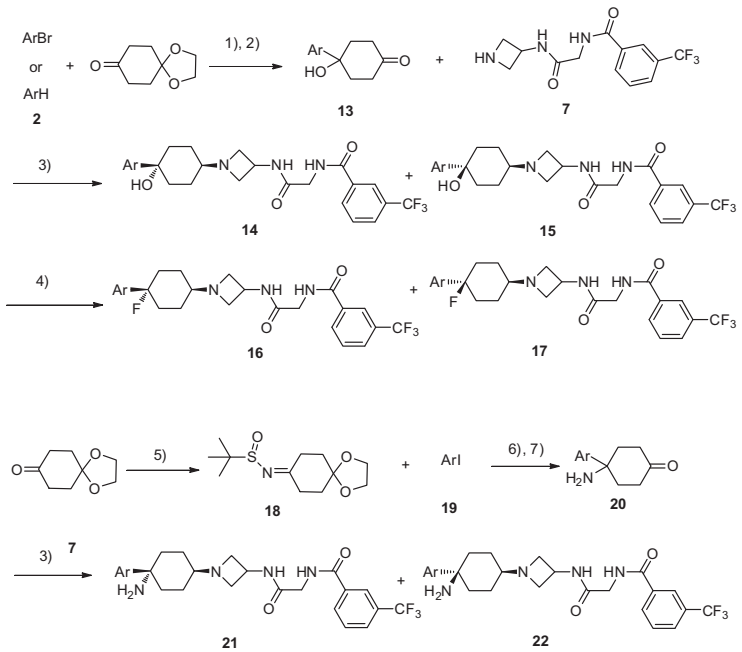
14a
CCR2 binding IC_{50}, 190 nM
hERG binding IC_{50}, 35 μM
hERG Patch, 22% @ 3 μM

Figure 3. Hydroxy azetidine.

As illustrated in Table 4, installation of OH group at the 1-position of the cyclohexanyl ring (**14b**) still maintained good CCR2 activity (IC_{50}, 36 nM) but reduced five folds of hERG binding affinity. Moreover, compound **14b** presented relatively clean hERG functional activity with only 34% inhibition at 3 μM. Other substituents of either F or NH_2 resulted in significant loss of CCR2 activity as shown in **16a** and **21a**. Incorporation of a cynao, carboylic acid or carbinol group (**23**–**25**) abolished CCR2 activity.

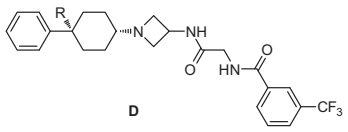
Substituent effect on the phenyl ring of **14b** was further examined (Table 5). Similar to their des-oxy analogs the electronic effect of the substituents on the left-end aryl ring had great impact on CCR2 activity. Electron donating groups such as methoxy (**14f**), dimethylamino (**14e**), methylenedioxy (**14g**) and dihydrofuran (**14h**) all improved CCR2 binding and functional activities. Electron-withdrawing groups such as F and CN caused at least 10-fold drop of CCR2 binding affinity. Steric hinderance seemed to abandon CCR2 activity as compound **14i** with 2,6-dimethyl substitution on the phenyl ring was inactive. In terms of hERG profile, hydroxy azetidine **14e** was particularly promising with hERG binding affinity of 25 μM and low hERG blockade in the functional assay (22% inhibition at 3 μM; solvent background, 15%). We were pleased to establish a large in vitro cardiovascular safety margin against hERG finally.

In summary, we have identified a novel series of CCR2 antagonists based on the azetidine scaffold. The discovery process is highlighted by divergent SARs on CCR2 and hERG activities, which enabled us to dial out hERG affinity in the scaffold and generated



Scheme 2. Reagents and conditions: (i) *n*-BuLi, 78 °C, then **2**; (ii) HCl, acetone; (iii) $NaBH(OAc)_3$; (iv) DAST, −78 °C; (v) *t*-BuS(O)NH_2, $Ti(OEt)_4$, (vi) *i*-PrMgBr, **19**; (vii) HCl, THF.

Table 4

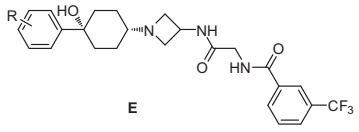


D

ID	R	CCR2 binding IC_{50}[a] (nM)	CTX IC_{50} (nM)	hERG binding IC_{50} (μM)	hERG patch% @ 3 μM
8b	H	35	35	2.0	97
14b	OH	36	55	10	34
16a	F	110	170	3.3	nt
21a	NH_2	240	160	6.2	nt
23	CN	>25,000[b]	nt	nt	nt
24	COOH	>25,000[b]	nt	nt	nt
25	CH_2OH	>25,000[b]	nt	nt	nt

[a] IC_{50} value are reported as the average of at least two separate determinations.
[b] IC_{50} values are reported by single determination.

Table 5



E

ID	R	CCR2 binding IC_{50}[a] (nM)	CTX IC_{50} (nM)	hERG binding IC_{50} (μM)	hERG patch%@ 3 μM
14b	H	36	55	10	34
14c	4-F	210	nt	2.8	nt
14d	3-CN	410	nt	1.5	nt
14e	3-NMe_2	15	22	25	22
14f	4-OMe	20	67	8.9	37
14g	3,4-Methylenedioxy	22	27	13.5	33
14h	3,4-Dihydrofuran	25	52	11.7	41
14i	2,6-Di-Me	>25,000[b]	nt	7.6	nt

[a] IC_{50} value are reported as the average of at least two separate determinations.
[b] IC_{50} values are reported by single determination.

promising selectivity for CCR2 over hERG which is likely to lead to a suitable cardiovascular safety margin.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.06.080.

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36. *MCP-1 receptor binding assay in THP-1 cells*:
THP-1 cells were obtained from American Type Culture Collection (Manassas, VA, USA). The THP-1 cells were grown in RPMI-1640 supplemented with 10% fetal bovine serum in a humidified 5% CO_2 atmosphere at 37 °C. The cell density was maintained at 0.5·106 cells/mL. THP-1 cells were incubated with 0.5 nM 125I labeled MCP-1 (Perkin–Elmer Life Sciences, Inc., Boston, MA) in the presence of varying concentrations of either unlabeled MCP-1 (R&D Systems, Minneapolis, MN) or test compound for 2 h at 30 °C in a 96 well plate. Cells were then harvested onto a filter plate, dried, and 20 μL of Microscint 20 was added to each well. Plates were counted in a TopCount NXT, Microplate

Scintillation & Luminescence Counter (Perkin–Elmer Life Sciences, Inc., Boston, MA). Blank values (buffer only) were subtracted from all values and drug treated values were compared to vehicle treated values. 1 μM cold MCP-1 was used for nonspecific binding.

37. *MCP-1 induced chemotaxis in THP-1 cells*:
MCP-1 induced chemotaxis was run in a 24-well chemotaxis chamber. MCP-1 (0.01 μg/mL) was added to the lower chamber and 100 μL of THP-1 cells (1×10^7 cell/mL) was added to the top chamber. Varying concentrations of test compound were added to the top and bottom chambers. Cells were allowed to chemotax for 3 h at 37 °C and 5% CO_2. An aliquot of the cells which had migrated to the bottom chamber was taken and counted then compared to vehicle.
38. *hERG [^{3}H]-astemizole binding experiment*: This assay is a 384well in-plate vacuum filtration binding assay. Assay reagents are added into a prepared/blocked 384well assay plate in the following order: (1) hERG Membrane diluted in Assay Buffer; (2) Test Compound; (3) ^{3}H Astemizole diluted in Assay Buffer. Assay reagents are incubated in the filter plate for 1 hour and then washed 6× with ice-cold wash buffer. Plates are allowed to dry overnight at room temperature. The following morning, plates are sealed and scintillant is added to each well. Following a two-hour incubation with scintillant, plates are placed on the TopCount and counted 1 min per well. Data is calculated using raw CPM. Where applicable, IC_{50} values are calculated using raw CPM values. Curves are fitted individually from singlet 11 point dosing curves + 1% DMSO control.
39. *Patch express experiment*: Experiments were performed using HEK293 cells stably expressing the HERG potassium channel. Cells were grown at 37 °C and 5% CO_2 in culture flasks in MEM Medium supplemented with 10% heat-inactivated fetal bovine serum, 1% L-Glutamine–Penicillin–Streptomycin-solution, 1% non-essential amino acids (100×), 1% sodium pyruvate (100 mM) and 0.8% Geneticin (50 mg/ml). Before use the cells were subcultured in MEM medium in the absence of 5 ml L-Glutamine–Penicillin–Streptomycin. For use in the automated patch-clamp system PatchXpress 7000A (Axon Instruments) cells were harvested to obtain cell suspension of single cells. Extracellular solution contained (mM): 150 NaCl, 4 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 HEPES, 5 Glucose (pH 7.4 with NaOH). Pipette solution contained (mM): 120 KCl, 10 HEPES, 5 EGTA, 4 ATP-Mg_2, 2 $MgCl_2$, 0.5 $CaCl_2$ (pH7.2 with KOH). Patch-clamp experiments were performed in the voltage-clamp mode and whole-cell currents were recorded with an automated patch-clamp assay utilizing the PatchXpress 7000A system (Axon Instruments). Current signals were amplified and digitized by a Multiclamp amplifier, stored and analyzed by using the PatchXpress, DataXpress software and Igor 5.0 (Wavemetrics). The holding potential was −80 mV. The HERG current (K^+-selective outward current) was determined as the maximal tail current at −40 mV after a 2 s depolarization to +60 mV. Pulse cycling rate was 15 s. Before each test pulse a short pulse (0.5 s) from the holding potential to −60 mV was given to determine (linear) leak current. After establishing whole-cell configuration and a stability period, the vehicle was applied for 5 min followed by the test substance by increasing concentrations of 3×10^{-6} M, 10^{-5} M and 3×10^{-5} M. Each concentration of the test substance was applied twice. The effect of each concentration was determined after 5 min as an average current of three sequential voltage pulses. To determine the extent of block the residual current was compared with vehicle pre-treatment. Data are presented as mean values ± standard error of the mean (SEM).
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