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Synthesis and biological evaluation of 3-aminopyrrolidine derivatives as CC chemokine receptor 2 antagonists

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ABSTRACT

Novel 3-aminopyrrolidine derivatives were synthesized and evaluated for their antagonistic activity against human chemokine receptor 2. Structure–activity studies on 3-aminopyrrolidine incorporating heteroatomic carbocycle moieties led to piperidine compound **19**, and piperazine compounds **42**, **47** and **49** as highly potent hCCR2 antagonists.

Monocyte chemoattractant protein-1 (MCP-1) is one of the chemokines of CC family and has been known to play an important role in the upregulation of immune cells in the biological system.[1,2] CCR2 (CC chemokine receptor 2), a receptor of MCP-1, belongs to the G-protein coupled receptor (GPCR) family, and the blocking of the interaction between MCP-1 and CCR2 results in variation of physiological activities including the abatement of inflammation. It has been suggested that antagonism mediated by monoclonal antibody or small molecule could be applied to the development of pharmaceutical candidates targeting on various inflammation-related diseases.[3–7] Rheumatoid arthritis, atherosclerosis, multiple sclerosis and type 2 diabetes mellitus have been tried as interesting therapeutic areas, and many literatures revealed the proof of concept through clinical trials and animal studies. Recently, diverse scaffolds as small molecule CCR2 antagonists have been explored mainly by pharmaceutical companies. Some examples are shown in Figure 1.[8–10]

Research groups of several pharmaceutical companies have been interested in 3-aminopyrrolidine derivatives substituted with glycine-linked 3-trifluoromethylbenzamide such as compound **1**. Centering around the pyrrolidine structure, derivatization of 3-(*R*)-substituted moiety showed rather restricted results. Instead of the glycine spacer, introduction of various α-substituted amino acids got rid of the CCR2 antagonism. Likewise, the substitution on the benzene ring failed to provide improved in vitro efficacy compared with the prior structure, except for 2-amino-5-trifluoromethyl substitution.[11] We synthesized related compounds such as 2,2-dimethylglycine adduct, trifluoromethylbenzenesulfonamide, and *N*-alkylamide derivatives. These compounds showed poor in vitro activity as CCR2 antagonist. Therefore, we focused on the variation of N-substitution of pyrrolidine. We here report their in vitro activities and properties as CCR2 antagonists, including the structure–activity relationship. The representative structures are shown in Figure 2.

Synthetic method of these compounds is shown in Schemes 1 and 2.[12] A major intermediate **9** could be easily prepared by amidation with glycine, followed by the amide coupling with benzyl-protected pyrrolidine and deprotection with hydrogenolysis using palladium catalyst. On the purpose to synthesize **5** and **6** series compounds, we prepared separately *N*-Boc-protected piperidine and piperazine derivatives that contained mesylated alcohol group as a leaving group linked with ethylene. After N-pyrrolidine substitution reaction, deprotection under acidic condition of Boc group was performed to prepare useful intermediates **10**. The obtained piperidine and piperazine moieties could be easily derivatized with reactions by use of benzoyl halides, benzyl halide, alkyl halide or aryl isocyanate to offer final compounds **11**.

We tried to introduce some small-sized alkyl side chains on ethylene linker, based on the idea that the ethylene linker between

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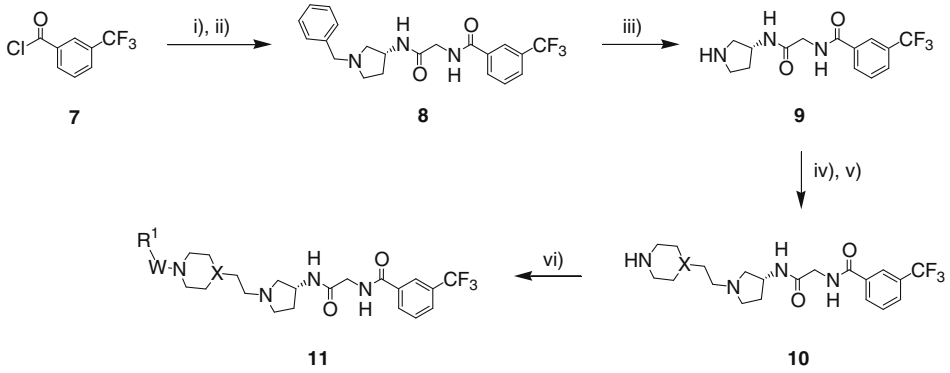
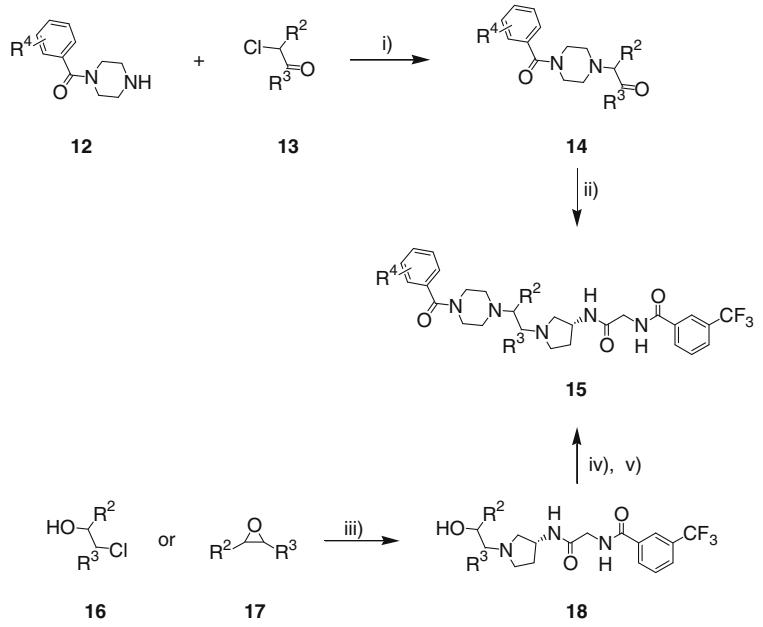


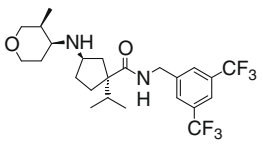
Scheme 1. Synthesis of piperidine and piperazine analogs. Reagents and conditions: (i) glycine, 2 N NaOH aq, CH_3CN; (ii) (3*R*)-1-benzyl-3-aminopyrrolidine, NMM, isobutyl chloroformate, THF; (iii) H_2 gas, $Pd(OH)_2$ cat., MeOH; (iv) 4-(2-methanesulfonyloxyethyl)-*N*-Boc-piperidine or 4-(2-methanesulfonyloxyethyl)-*N*-Boc-piperazine, K_2CO_3, CH_3CN; (v) HCl satd EtOH; (vi) any proper reagents to prepare final products.



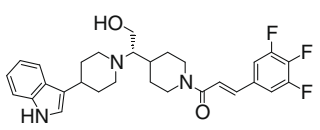
Scheme 2. Synthesis of piperazine analogs. Reagents and conditions: (i) K_2CO_3, CH_3CN; (ii) compound **9**, $NaBH(OAc)_3$, THF; (iii) compound **9**, K_2CO_3, CH_3CN; (iv) methanesulfonyl chloride, TEA, MC; (v) compound **12**, K_2CO_3, CH_3CN.

1 : Teijin
1a : R = 4-Cl, IC_{50} = 180nM (CCR2 binding)
1b : R = 2,4-di-Me, IC_{50} = 54nM (CCR2 binding)

2 : BMS
IC_{50} = 5.1nM (CCR2 binding)

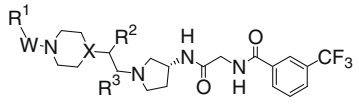


3 : Merck
IC_{50} = 1.2nM (CCR2 binding)
IC_{50} = 0.1nM (chemotaxis)



4 : J&J
IC_{50} = 2.4nM (CCR2 binding)

Figure 1. CCR2 antagonists.

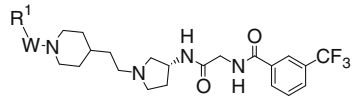


5 : X = C
6 : X = N

W = CO, SO_2, CH_2
R^1 = aryl
R^2, R^3 = H, alkyl

Figure 2. 4-Piperidinoethyl and piperazinoethyl pyrrolidine analogs.

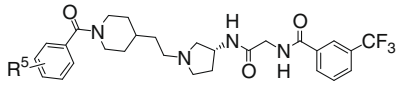
Table 1
Evaluation of piperidine derivatives



Compound	R^1	W	IC_{50} (μM)		
			hCCR2b binding[a]	Ca^{2+} flux	Chemotaxis
19	Ph	C=O	0.58	0.030	0.023
20	3-Pyridyl	C=O	56%		
21	4-Pyridyl	C=O	53%		
22	2-Naphthyl	C=O	21%		
23	Ph	CH_2	15%		
24	Ph	SO_2	41%		
25	*n*-Butyl	C=O	32%		
26	PhO–	C=O	39%		
27	PhNH–	C=O	22%		
1a[b]			3.20	0.98	>0.30
1b[b]			0.61	0.30	0.070

[a] % inhibition at 10 μM when no IC_{50} was measured.
[b] Reference compounds (Fig. 1).

Table 2
Evaluation of *N*-benzoyl piperidine derivatives

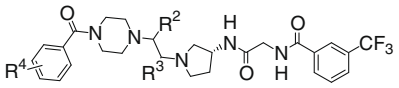


Compound	R^5	IC_{50} (μM)		
		hCCR2b binding[a]	Ca^{2+} flux[b]	Chemotaxis
28	2-F	1.13	0.029	>0.10
29	3-F	8.33	0.16	
30	4-F	6.60	25%	
31	2,5-Di-F	1.41	0.011	>0.10
32	2-Cl	1.31	0.012	0.009
33	4-Cl	3.16	31%	
34	3,4-Di-Cl	7.52	41%	
35	4-CF_3	2.21	0.66	
36	2-Me	2.88	0.52	
37	3-Me	1.18	0.75	
38	4-Me	1.00	0.62	
39	4-Et	1.74	47%	
40	4-*n*-Pr	1.73	35%	
41	3,5-Di-Me	48%		

[a] % Inhibition at 10 μM when no IC_{50} was measured.
[b] % Inhibition at 1 μM when no IC_{50} was measured.

two ring systems might tolerate some hydrophobic group substitution. Moreover, it was expected to provide steric constraint to flexible ethylene linker. This idea was also supported by the in-house modeling study (data not shown). Synthesis of these compounds

Table 3
Evaluation of piperazine derivatives



Compound	R^2	R^3	R^4	IC_{50} (μM)		
				hCCR2b binding	Ca^{2+} flux	Chemotaxis
42	H	H	H	0.99	0.018	0.009
43	H	H	2-Me	4.44	0.12	
44	H	H	3-Me	3.52	0.17	
45	H	H	4-Me	3.46	0.11	
46	H	H	4-F	>10	1.10	
47	H	H	2-Cl	1.12	0.0017	0.023
48	H	H	2-F	1.88	0.0026	>0.10
49	H	H	2,5-Di-F	3.04	0.0037	0.010
50	Me	H	H	>10	0.71	
51	H	Me	H	>10	1.86	
52	H	Et	H	>10	>10	
53	Et	H	H	>10	>10	

was carried out by two methods as shown in Scheme 2. Starting from commercially available or in-house synthesized reagents, various ketone analogs **14** were made, and reductive amination with intermediate **9** using mild reductive agent such as sodium triacetoxyborohydride offered the target compounds **15**. Additionally, chloroethanol derivatives or 2-/3-mono substituted epoxide reagents were used to get our final compounds **15** via mesylated alcohol intermediates. In these cases, proper synthetic methods were chosen according to ease-of-synthesis.

Primary human CCR2b (hCCR2b) protein binding inhibitory activity of all the synthesized compounds was monitored against human [^{125}I]MCP-1 binding to THP-1 cells.[13] The functional activity of selected compounds was then measured using the calcium flux (Flexstation, HEK293/CCR2b cells) and chemotaxis (human MCP-1, monocytes) inhibition assays.[14,15] To compare the activity of our compounds with previously published compounds, *N*-benzylpyrrolidine derivatives **1a**, **1b** which contain similar structure with our compounds were co-assayed as the references. In the hCCR2b protein binding experiments, the reference compounds showed weak inhibition compared to their activities reported previously. But in the cell-based functional assays, they showed the close level of activities to literature data. Therefore we integratively evaluated the activities of the synthesized compounds from three in vitro assays, inhibition of protein binding affinity, calcium flux and chemotaxis.

Table 4
Evaluation of drug candidates

Compound	IC_{50} (μM)			Cytotoxicity[d] (GI_{50}, μM)
	hERG K[a]	CYP450[b]	GPCR[c]	
19	>10	>10	>10	>10
28	>10	>10[e]	>10	>10
31	≒10	>10[e]	>10	>10
32	>10	>10[e]	>10	>10
42	>10	>10	>10	>10
47	>10	>10	>10	>10
48	>10	>10	>10[f]	>10
49	>10	>10	>10	>10

[a] Human ERG potassium channel binding inhibition/[^{3}H]astemizole.
[b] Human recombinant enzyme: 1A2, 2C9, 2C19, 2D6 and 3A4 by P450-Glo method.
[c] Receptors: 5-HT1a, 5-HT2a, 5-HT2c, 5-HT6, 5-HT7, D2, D3 and D4.
[d] Test cell line: HepG2, NIH 3T3, CHO-K1, HEK 293 and HUVEC by MTT assay.
[e] Except for 3A4 subtype.
[f] Except for 5-HT6 and D4.

As shown in Table 1, introduction of various N-substituents of ethylene-linked piperidine resulted in the validity of benzoyl group, compound **19**, although alkylcarbonyl, heteroarylcarbonyl, benzenesulfonyl, arylcarbonate and arylcarbamate groups showed poor inhibition activity. In optimizing the substituents on the benzoyl group of compound **19** (Table 2), halogenated derivatives **28**, **31** and **32** showed comparable or slightly improved activities in the functional calcium flux assay, and compound **32** was proved to be the most active among them in the chemotaxis assay (IC_{50} = 9 nM). However, alkyl substitution lowered the activity of compounds regardless of the site on benzene ring. Furthermore, as the alkyl groups became bulkier, the compounds were less active. After all our effort of the replacement of other various functional groups, it is evident that polar or bulky nonpolar substituents were limited. For the halogen substitution, 2-position was preferred, while 4-position was slightly preferred for methyl substitution.

Further optimization was carried out on the piperidine ring, replacing 4-carbon with nitrogen to offer piperazine analogs (Table 3). In the calcium flux and chemotaxis assay, compound **42** resulted in improved activity compared with compound **19**. As is the case of piperidine series, halogenation on the benzoyl group enhanced the functional activities. In the calcium assay compound **47**, **48** and **49** showed excellent inhibitory activity (IC_{50} = 1.7, 2.6 and 3.7 nM, respectively), and compound **47** and **49** have also good potency in the chemotaxis assay (IC_{50} = 23 and 10 nM, respectively). The presence of small alkyl side chain on ethylene linker did not, however, yield more active compounds. Despite the possibility of spacial margin in the protein binding based on the comparison with published similar molecules and our molecular modeling study, the introduction of alkyl group on the ethylene linker probably disturbed the effective interaction of benzoylpiperazine moiety in the binding site of CCR2 protein.

Selected compounds which were found to be active in the binding and the functional assays were evaluated further using several in vitro assays (Table 4). In the human ERG potassium inhibition assay and the cytotoxicity test, no toxicity was observed for most compounds. However, halogenated analogs, **28**, **31** and **32**, exhibited moderate inhibitory activities in CYP450 enzyme assay (IC_{50} = 0.3–0.8 μM for 3A4 subtype). Selectivity of compounds for other GPCRs was initially determined in 5-HT and dopaminergic receptors. All compounds showed no significant binding, except for compound **48** which slightly bound to 5-HT6 and D4 receptors (IC_{50} = 3.6 and 3.8 μM, respectively).

In conclusion, new series of ethylene-linked piperidine and piperazine-3-aminopyrrolidine derivatives were synthesized. Piperidine compound **19** and piperazine compounds **42**, **47** and **49** possessed excellent functional activities related to hCCR2 antagonism. Various pharmacological assays demonstrated the usefulness of these compounds as drug candidates. Further studies are being conducted to characterize the in vivo efficacy and pharmacokinetics of these series of hCCR2 antagonists.

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12. All final compounds displayed spectral data (NMR, MS) that were consistent with the assigned structures.
13. Membranes from stable HEK293-EBNA cell line expressing the human chemokine CCR2b receptor (PerkinElmer Life and Analytical Sciences, Boston, USA) were used. For CCR2b receptor binding assay, cell membrane (8 μg/well), 0.03 nM [^{125}I]-MCP-1 (PerkinElmer) and appropriate concentrations of test compounds were added to 0.25 ml of 25 mM Hepes (pH 7.2) buffer containing 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.2% BSA. The mixture was incubated for 60 min at 27 °C, and the reaction was terminated by rapid filtration using a cell harvester (Inotech, Switzerland) through Filtermat A GF/C glass fiber filter presoaked in 0.3% polyethylenimine. The filter was covered with MeltiLex, sealed in a sample bag followed by drying in a microwave oven, and counted by MicroBeta Plus (Wallac, Finland). Nonspecific binding was determined in the presence of 0.05 μM human MCP-1. Competition binding studies were carried out with 7–8 varied concentrations of the test compounds run in duplicate tubes, and isotherms from three assays were calculated by computerized nonlinear regression analysis (GraphPad Prism Program, San Diego, USA) to yield inhibition values (IC_{50}).
14. FlexStation (Molecular Devices, Inc.) was used to measure the intracellular calcium concentration in HEK293/CCR2b cells. The cells were seeded at 40,000 cells/well in a 96-well, black-wall, clear-bottom, tissue culture treated polystyrene plate (Costar 3603, Corning, NY) and grown at 37 °C in an incubator with 5% CO_2 for 24 h. After removing the media completely, the cells were dye-loaded with Fluo-4 NW (Invitrogen) containing 1.25 mM probenecid for 50 min at 37 °C. Subsequently, the cells were treated with various concentrations of compounds and incubated further for 10 min at 37 °C. Activation of CCR2b receptor was achieved by the addition of MCP-1 (final concd = 20 nM) to the cells and the changes in relative fluorescence unit due to the increase in the intracellular calcium concentration were monitored in the FlexStation.
15. For a description of the chemotaxis assay, see: Han, K. H.; Tangirala, R. K.; Green, S. R.; Quehenberger, O. *Arterioscler. Thromb. Vasc. Biol.* **1998**, *18*, 1983.