Discovery of Disubstituted Cyclohexanes as a New Class of CC Chemokine Receptor 2 Antagonists

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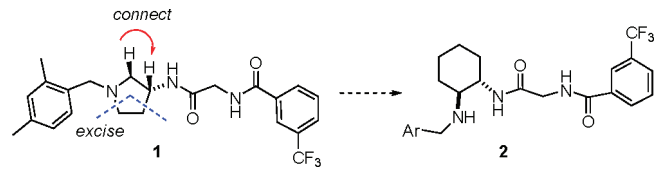
Abstract: We describe the design, synthesis, and evaluation of novel disubstituted cyclohexanes as potent CCR2 antagonists. Exploratory SAR studies led to the *cis*-disubstituted derivative **22**, which displayed excellent binding affinity for CCR2 (binding $IC_{50} = 5.1$ nM) and potent functional antagonism (calcium flux $IC_{50} = 18$ nM and chemotaxis $IC_{50} = 1$ nM). Site-directed mutagenesis studies with **22** suggest the compound is binding near the key receptor residue Glu291, however, **22** is not reliant on Glu291 for its binding affinity.

Chemokines are small molecular weight proteins implicated in inflammatory and autoimmune processes through their role as leukocyte chemoattractants.[1] To date, over 50 chemokines have been identified, with the majority belonging to two major families (CC and CXC).[2] We have been interested in a CC chemokine family member, monocyte chemoattractant protein-1 (MCP-1[a] or CCL2),[3] which is produced by a variety of cells in response to inflammatory stimuli. MCP-1 elicits a functional response by binding to its receptor, CC chemokine receptor 2 (CCR2), which is a member of the G-protein coupled receptor family (GPCR).[4] The CCR2/MCP-1 pair plays a major role in the activation and migration of monocytes from the circulation to sites of inflammation within the tissues. Both MCP-1 and CCR2 have been implicated in a host of diseases that are characterized by monocyte accumulation, including rheumatoid arthritis,[5] atherosclerosis,[6] and multiple sclerosis,[7] and insulin resistance.[8] As a result, there has been much interest in the design and synthesis of CCR2 antagonists as potential therapeutics.[9] In this communication, we describe the discovery of disubstituted cyclohexane derivatives as novel and potent CCR2 antagonists.

As members of the GPCR family, chemokine receptors contain the common 7-transmembrane (7-TM) core as the main structural unit. However, chemokine receptors are unique in that they contain a conserved glutamic acid (Glu) in TM7.[10] In CCR2, this is Glu291, which has been shown to be critical for small molecule antagonist binding via site-directed mutagenesis.[11] Included in this research[11a] was a Teijin/CombiChem compound **1**,[12] which was shown to have a large reliance on Glu291 for CCR2 binding, presumably through an interaction with the basic pyrrolidine nitrogen. We became interested in the binding of **1** and considered several binding modes. In one case, we hypothesized that the two highlighted hydrogens (see Scheme 1) of the pyrrolidine might project toward solvent, hence, identifying an area that could accommodate a new constraining factor. If a cyclohexane was selected as the constraining factor, and the pyrrolidine ring was excised along the dotted lines, this would reveal a *trans*-1,2-disubstituted cyclohexane **2**. As shown by the overlay of **1** and **2** (Figure 1), the two basic amines come in close proximity to one another; however, the benzyl component appears to be in need of optimization.

Scheme 1



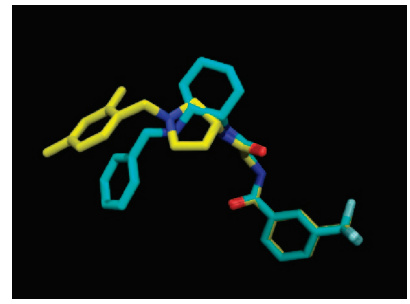


Figure 1. Overlay of Teijin/CombiChem derivative **1** (yellow) and compound **2** (cyan).

To test our hypothesis, we evaluated several *trans*-disubstituted cyclohexanes using a radiolabeled MCP-1 displacement assay in peripheral blood mononuclear cells (PBMCs) to assess their binding affinity for CCR2 (see Table 1).[13] We were interested in selective CCR2 antagonists and, hence, used a CCR3 binding assay[14] for an initial assessment of selectivity over a chemokine family member. The *trans*-disubstituted core **2** did show activity in the CCR2 binding assay as the 2,4-dimethyl derivative **3** had a CCR2 IC_{50} of 0.54 μM and displayed good selectivity versus CCR3. However, conversion of the secondary amine to the more basic tertiary amine **4** was not advantageous for the CCR2 affinity, contrary to what one would expect for a pyrrolidine mimetic. Modifying the 2,4-dimethyl substitution pattern was met with some difficulty, as the addition of a third methyl to the 2,4,6-derivative **5** gave a 2-fold loss in activity relative to **3**. Likewise, the 2,4-dichloro derivative **6** and the 4-chloro derivative **7** both lost affinity for CCR2 as compared to **3**. Not surprisingly, the amide **8** lost substantial affinity (12-fold) for CCR2 when compared to **7**.

We focused our optimization on the benzylamine portion of the molecule and next explored the racemic *cis*-disubstituted cyclohexanes shown in Table 2. The *cis*-2,4-dimethyl derivative **9** was 4-fold less active for CCR2 relative to the same *trans*-derivative **3**. However, exploration of monosubstitution at the 4-postion was promising, as several derivatives (**10**−**13**) displayed good affinity for CCR2 with promising (~20-fold) selectivity versus CCR3. The best compound of the series was the 4-isopropyl derivative **13** with a CCR2 binding IC_{50} of 0.36 μM.

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[a] Abbreviations: MCP-1, monocyte chemoattractant protein-1; CCL2, chemokine (C-C motif) ligand 2; CCR-2, CC chemokine receptor 2; GPCR, G-protein coupled receptor; 7-TM, 7-transmembrane; CTX, chemotaxis.

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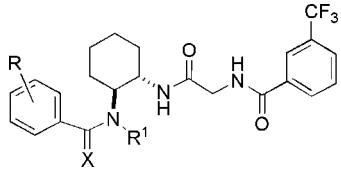
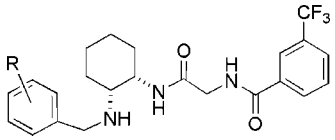
Table 1. Evaluation of *trans*-Disubstituted Derivatives



cmpd	R	X	R^1	CCR2 binding IC_{50}[a] (μM)	CCR3 binding % inh at 10 μM[b]
3	2,4-diMe	H,H	H	0.54 ± 0.24 (2)	54%
4	2,4-diMe	H,H	Me	3.01 ± 3.14 (2)	NT[c]
5	2,4,6-triMe	H,H	H	1.19 ± 0.84 (2)	NT
6	2,4-diCl	H,H	H	7.86 ± 1.78 (2)	NT
7	4-Cl	H,H	H	0.97 ± 0.65 (3)	50%
8	4-Cl	O	H	12 (1)	NT

[a] IC_{50} values (n) are displayed as mean ± SD ($n = 2$) and mean ± SEM ($n > 2$). [b] CCR3 % inhibition are $n = 1$. [c] NT = not tested.

Table 2. Evaluation of *cis*-Disubstituted Derivatives[a]



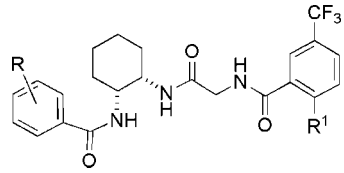
cmpd	R	CCR2 binding IC_{50}[b] (μM)	CCR3 binding % inh at 10 μM[c]
9	2,4-diMe	2.17 (1)	54
10	4-Cl	0.79 ± 0.46	24
11	4-OCF_3	0.53 ± 0.0042	50
12	4-CF_3	0.40 ± 0.096	47
13	4-*i*-Pr	0.36 ± 0.20	55

[a] Compounds are racemic, one enantiomer is displayed for illustrative purposes. [b] IC_{50} values are displayed as mean ± SD ($n = 2$) [c] CCR3 % inhibition are $n = 1$.

As in the *trans*-series, we subsequently made *cis*-amides (see Table 3), and compound **14** displayed weak CCR2 activity (CCR2 binding IC_{50} = 4.13 μM). Surprisingly, the 4-methyl derivative **15** provided a 7-fold gain in affinity, and the 4-iodo derivative **16** gave a 100-fold increase in affinity for CCR2 when compared to **14**. As a result of this activity, compound **16** was tested in the calcium flux assay (a functional assay based in PBMCs)[13] and gave an IC_{50} of 30 nM in the presence of MCP-1. Calcium flux inhibition was unchanged when the incubation with **16** was performed without MCP-1 (in the presence of **16** only), thus validating compound **16** as an antagonist.[15] Compound **16** was also tested in the chemotaxis assay[13] and it was found to have good activity (CTX IC_{50} = 160 nM). Continuing at the 4-position, both the 4-thiomethyl derivative **17** and the 4-sulfonylmethyl derivative **18** lost affinity for CCR2, whereas the sulfonamide derivative **19** gained some binding affinity as compared to **16**. To achieve additional affinity, we turned our attention to the 2-position of the glycinamide benzamide.[12] The 2-amino compound **20** did not enhance the binding affinity for CCR2 as compared to **19**, but it did improve the potency in the chemotaxis assay by 2-fold and the calcium assay by 8-fold relative to **19**. In fact, we found that large groups in this position could have a dramatic enhancing effect on chemotaxis potency.[16] For example, the cyclohexyl amide derivative **21** had a 2-fold increase in CCR2 binding affinity as compared with **20**, but its chemotaxis potency increased 126-fold.[17] Likewise, the urea derivative **22** had a 4-fold increase in binding affinity relative to **20**, while its chemotaxis potency increased by 63-fold.

As nonbasic antagonists, these compounds are rare in the CCR2 field,[18] given the previously mentioned reliance on Glu291 for antagonist binding.[10,11] To probe the reliance of our compounds for Glu291 and obtain information on their placement within the receptor, we investigated the binding of three compounds with single-site receptor mutants of CCR2 (Table 4). The first receptor mutant studied was the well characterized Glu291 to Ala291 (E291A).[19] Pyrrolidine **1** had a large reliance on Glu291 for binding and showed a 100-fold shift in binding affinity between wild-type and the E291A mutant.[11a] Our benzyl amine compound **10** displayed a negligible change in binding affinity (8-fold) when compared in wild-type and the E291A mutant, as did the amide **14**. Our most potent compound **22** was tested in the E291A mutant and it did not exhibit a shift in the binding affinity, indicating that even **22** had no reliance on Glu291 for CCR2 binding. Hence, the *cis*-amides did not have a reliance on Glu291. As Glu291 is conserved across the chemokine receptors, we felt this was a positive step toward achieving selectivity over other chemokine family members. It also differentiated our compounds from pyrrolidine **1**, however, it did not offer any information as to the placement of our compounds within the receptor. For this reason, we also tested compound **22** in a second mutant Thr292 to Ala292 (T292A), which is the residue adjacent to Glu291. This mutant was previously described,[11a] and pyrrolidine **1** was reported to have a 30-fold binding shift when tested in the T292A mutant as

Table 3. Evaluation of *cis*-Diamide Derivatives[a]



cmpd	R	R^1	IC_{50}[b] (μM) CCR2 binding	Ca flux	CTX mono	CCR3 binding % inh at 10 μM[c]
14	2,4-diMe	H	4.13 (1)	NT[d]	NT	NT
15	4-Me	H	0.55 ± 0.16 (2)	NT	NT	57
16	4-I	H	0.037 ± 0.0071 (2)	0.030 (1)	0.16 ± 0.0081 (2)	27
17	4-SMe	H	0.064 ± 0.023 (2)	0.215 ± 0.035 (2)	1.2 (1)	31
18	4-SO_2Me	H	0.087 ± 0.051 (2)	NT	NT	3
19	4-SO_2NH_2	H	0.021 (1)	0.042 ± 0.0028 (2)	0.13 ± 0.16 (2)	39
20	4-SO_2NH_2	NH_2	0.023 ± 0.0045 (6)	0.0048 ± 0.0011 (4)	0.063 ± 0.033 (2)	6
21	4-SO_2NH_2	$NHCOC_6H_{11}$	0.009 ± 0.0027 (6)	0.0037 ± 0.0017 (3)	0.0005 ± 0.0001 (4)	10
22	4-SMe	NHCONH*i*-Pr	0.0051 ± 0.0036 (2)	0.018 ± 0.00071 (2)	0.001 ± 0.0002 (2)	37

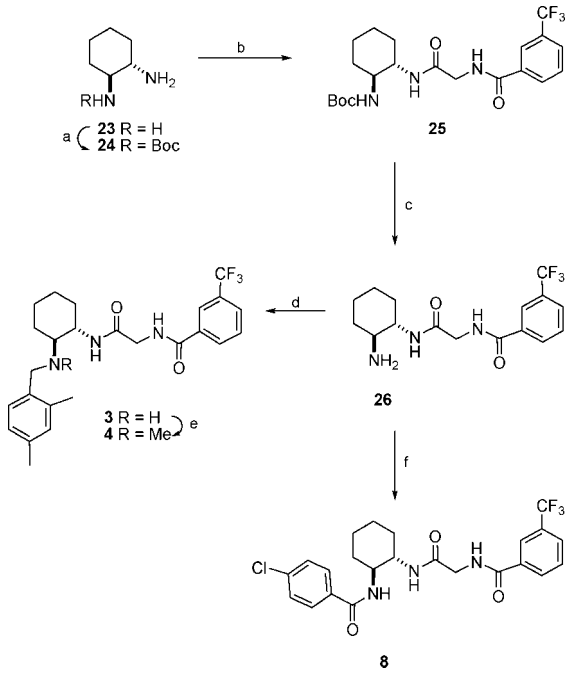
[a] Compounds are racemic, one enantiomer is displayed for illustrative purposes. [b] IC_{50} values (n) are displayed as mean ± SD ($n = 2$) and mean ± SEM ($n > 2$). [c] CCR3 % inhibition are $n = 1$. [d] NT = not tested.

Table 4. Evaluation of Derivatives Versus Mutant CCR2 Receptors

cmpd	WT CCR2 IC_{50}[a] (nM)	E291A IC_{50}[a] (nM)	E291A fold change	T292A IC_{50}[a] (nM)	T292A fold change
10	268 (1)	2117.2 (1)	7.9	NT[b]	NT
14	4130 ± 1089 (2)	7847 (1)	1.9	NT	NT
22	7.5 ± 1.9 (3)	3.7 (1)	0.5	303.7 (1)	40.5

[a] IC_{50} values (n) are displayed as mean ± SD ($n = 2$) and mean ± SEM ($n > 2$). [b] NT = not tested.

Scheme 2. Representative Synthesis[a]



[a] Reagents and conditions: (a) BOC-ON, dioxane; (b) BOP, NMM, (3-(trifluoromethyl)-benzamido) acetic acid, DMF, 0 °C; (c) TFA, CH_2Cl_2, 0 °C; (d) $(i\text{-Pr})_2$NEt, 2,4-dimethylbenzaldehyde, 4 Å MS, $NaBH(OAc)_3$, THF; (e) $(i\text{-Pr})_2$NEt, 37% formaldehyde, $NaBH(OAc)_3$, THF; (f) BOP, NMM, 4-chloro-benzoic acid, DMF, 0 °C.

compared to wild-type.[11a] Our examination of compound **22** in the T292A mutant showed a very similar 40-fold shift. Hence, although **22** did not utilize Glu291 in its binding, it did involve the adjacent residue T292. This may suggest our antagonists occupy a similar region of the receptor as that previously described.[11]

A representative synthesis of these analogues is shown in Scheme 2 for the compounds of Table 1. The purchased diamine **23** was selectively protected as the *tert*-butyl carbamate **24**.[20] The glycinamide side chain was then attached in one operation via a coupling with BOP reagent to give **25**. Carbamate removal gave **26** and subsequent reductive amination gave the final target **3**. The secondary amine was methylated via a second reductive amination with formaldehyde to give **4**. The amide **8** was produced from amine **26** via a standard BOP coupling. The compounds of Tables 2 and 3 were synthesized in an analogous manner starting with racemic *cis*-(1*R**,2*S**)-cyclohexane-1,2-diamine.

In summary, we have described the design, synthesis, and evaluation of novel disubstituted cyclohexane derivatives as potent CCR2 antagonists. SAR studies led to a series of nonbasic antagonists, which displayed potent activity in two functional assays. Exploring these antagonists further with site-directed mutagenesis, we verified their lack of dependence on the key receptor residue Glu291. Additional studies using the T292A mutant and compound **22** suggested a placement within the Glu291 pocket. Hence, the CCR2 affinity has been optimized for these compounds without utilizing an interaction to Glu291. This strategy has not been widely described, as nonbasic CCR2 antagonists are uncommon.

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Supporting Information Available: Compound characterization data for all target compounds and experimental details for the representative synthesis of Scheme 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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