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Synthesis and Biological Evaluation of 1-Cyclohexyl Substituted 3-Aminopyrrolidine Derivatives as CC Chemokine Receptor 2 (CCR2) Antagonists

Jee Woong Lim,[†,ℙ,*] Yongho Na,[†] Min-Ho Oak,[‡] Jong-Hoon Kim,[†,§] Youna Oh,[†] Seung-Woo Lee,[†] Heeyeong Cho,[#] Woo-Kyu Park,[#] Gildon Choi,[#] Kyoung Rak Kim,[†] and Jongmin Kang[ℙ,*]

[†]*Yangji Chemicals, Gyeonggi Bio-Center, Suwon 443-766, Korea.* [*]*E-mail: srhnys@hwpharm.com*
[‡]*Research and Development Center, Hanwha Pharma, Chuncheon 200-921, Korea*
[§]*Department of Biotechnology, Yonsei University, Seoul 120-749, Korea*
[#]*Korea Research Institute of Chemical Technology, Daejeon 305-600, Korea*
[ℙ]*Department of Chemistry, Sejong University, Seoul 143-747, Korea*
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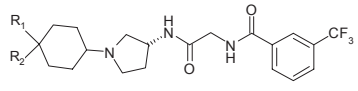
CC chemokine receptor 2 (CCR2), a receptor of monocyte chemotactic protein-1 (MCP-1), has been known to play an important role in the upregulation of immune cells in the biological system.[1] Over the last decade extensive preclinical studies, including various animal disease models, have uncovered a role of CCR2 in the inflammation-related diseases such as rheumatoid arthritis, multiple sclerosis, atherosclerosis, chronic obstructive pulmonary disease (COPD) and type 2 diabetes mellitus.[2] Recently several pharmaceutical companies performed phase I and II clinical trials with their own drug candidates as a CCR2 antagonist. Herein we report on the 1-cyclohexyl substituted 3-aminopyrrolidine derivatives (**1** and **2**) as potential drug candidates as CCR2 antagonists (Figure 1).

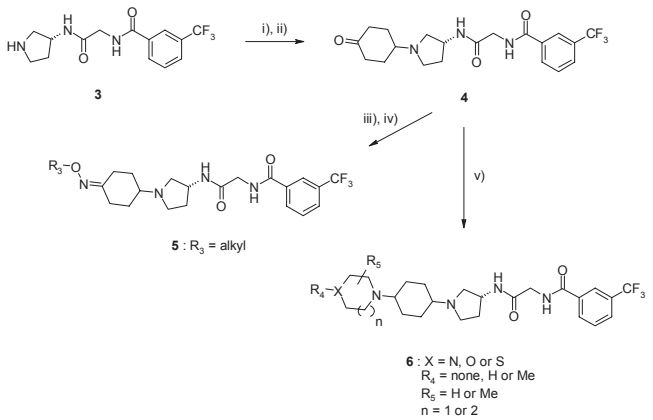
The research for the finding of lead compounds based on 3-aminopyrrolidine moiety was performed by several pharmaceutical companies. As a result of our work, there evidently exists no flexibility of derivatization at the 3-amino position of pyrrolidine to enhance the antagonistic activities toward CCR2 receptor, therefore we focused on the substitution at the 1-position of pyrrolidine. According to the in-house modeling study (data not shown), it is presumed that the binding environment of this 1-substituent of pyrrolidine on CCR2 protein would be hydrophobic to a certain degree. So we investigated some nonpolar groups such as small-sized alkyl chains and cycloalkanes, and found that cyclohexyl group was very effective to exhibit the binding affinity to CCR2. Next we examined various substituents on the 1,4-linked cyclohexyl group, mainly *N*-substituents including aliphatic amines, benzylamines, oximes, tetrahydroisoquinoline, pyrrolidine and several heteroatomic carbocycles. After the initial SAR studies, we found that *O*-alkyl ketoxime, morpholine and piperazine groups provided the validity to offer CCR2 antagonism with structural novelty. So we decided to modify these moieties to further enhance potency. Synthetic method of these compounds is shown in Scheme 1. Reductive amination of compound **3** with protected 1,4-cyclohexanedione followed by deprotection of the ketal gave major intermediate **4**.[3] This compound can be used as an intermediate to provide a path to two catagories of our target molecules, *O*-alkyl ketoxime **5** and heteroatomic carbocycle **6** derivatives of 1-cyclohexyl 3-aminopyrrolidine.[4]

Human CCR2b (hCCR2b) protein binding inhibitory activities of all the synthesized compounds were primarily monitored against human [^{125}I]MCP-1 binding to THP-1 cells, as summarized in Table 1. Among the *O*-alkyl ketoxime analogs (**5a-5e**), compound **5b** showed an optimum activity in the binding affinity assay, and the increased bulkiness of alkyl group lowered the activity. The introduction of morpholine or thiomorpholine (**6a-6e**) was found to be more effective than that of oxime. Specially 1,4-di-substitution of cyclohexane gave *cis* and *trans* isomers, which were separated by means of flash chromatography on silica gel. In all cases less polar isomers showed higher binding affinity than more polar isomers regardless of *N*-substituents. The cell-based functional activities of



1 : R_1 = R_2 = *O*-alkyl ketoxime
2 : R_1 = morpholine, thiomorpholine or piperazine
R_2 = H

Figure 1. 1-Cyclohexyl substituted 3-aminopyrrolidine derivatives.



Scheme 1. Synthesis of *O*-alkyl ketoxime analogs. Reagents and conditions : i) 1,4-cyclohexanedione monoethyleneketal, $NaBH(OAc)_3$, THF; ii) 3N-HCl aq., THF; iii) hydroxylamine hydrochloride, $NaHCO_3$, MeOH; iv) dialkyl sulfate, 2N-NaOH aq., MeOH; v) morpholine, thiomorpholine or piperazine reagent, $NaBH(OAc)_3$, methylene chloride

Table 1. Evaluation of 1-cyclohexyl substituted 3-aminopyrrolidine derivatives

compound	R_3	R_4	R_5	X	n	isomer	hCCR2 binding[a]	
							% inh.@10μM	IC_{50} (μM) ± SEM
5a	Me						52	5.14 ± 0.57
5b	Et						66	2.48 ± 0.25
5c	*n*-Pr						58	6.16 ± 0.18
5d	*n*-Bu						55	7.78 ± 1.31
5e	benzyl						46	> 10
6a		-	H	O	1	mixture	79	0.77 ± 0.15
6b		-	H	O	1	less polar	77	0.72 ± 0.19
6c		-	H	O	1	polar	28	> 10
6d		-	H	S	1	less polar	85	1.54 ± 0.72
6e		-	H	S	1	polar	20	> 10
6f		H	H	N	1	less polar	15	> 10
6g		H	H	N	1	polar	12	> 10
6h		Me	H	N	1	polar	21	> 10
6i		H	H	N	2	less polar	32	> 10
6j		H	H	N	2	polar	17	> 10
6k		H	2,5-di-Me	N	1	less polar	28	> 10
6l		H	2,5-di-Me	N	1	polar	26	> 10
BMS CCR2 22[b]							66	2.60 ± 0.22

[a]Data were obtained from two separate experiments. [b]Reference compound.[5]

Table 2. Functional activities of drug candidates

compound	IC_{50} (μM) ± SEM[a]	
	chemotaxis	Ca flux
5b	0.028 ± 0.0052	37%[b]
6b	0.048 ± 0.0085	0.430 ± 0.050
6d	0.025 ± 0.0061	0.270 ± 0.021
BMS CCR2 22	ND[c]	0.016 ± 0.004

[a]Data were obtained from two separate experiments. [b]% inhibition at 1 μM. [c]Not determined.

Table 3. Evaluation of drug candidates[a]

compound	PAMPA[b] (Log P_e ± SEM)	CYP450[c] (IC_{50}, μM)	GPCR[d] (IC_{50}, μM)	Cytotoxicity[e] (GI_{50}, μM)
6b	−5.94 ± 0.06	> 10	> 10	> 10
6d	−5.56 ± 0.06	> 10	> 10	> 10

[a]All data were obtained from two separate experiments except for PAMPA. [b]Data were obtained from five separate experiments (BD Gentest pre-coated PAMPA plate system). [c]Human recombinant enzyme: 1A2, 2C9, 2C19, 2D6 and 3A4 by P450-Glo method. [d]Receptors: 5-HT1a, 5-HT2a, 5-HT2c, 5-HT6, 5-HT7, D2, D3 and D4. [e]Test cell line: HepG2, NIH 3T3, CHO-K1, HEK 293, HUVEC and THP-1 by MTT assay.

selected compounds, **5b**, **6b** and **6d**, were next evaluated using the calcium flux (Flexstation, HEK293/CCR2b cells) and chemotaxis (human MCP-1, monocytes) inhibition assays. Signaling circuits enganged by chemokine receptors include calcium channel opening, and result in the movement of the cell. Therefore functional calcium flux and chemotaxis activities are essential in the assessment of CCR2 antagonism. As seen in Table 2, *O*-alkyl ketoxime **5b** had a weak calcium flux inhibitory activity. On the other hand, morpholine **6b** and thiomorpholine **6d** showed good activities both in chemotaxis and calcium flux assays, and especially thiomorpholine adduct was found to be slightly better than the morpholine derivative. Compounds **6b** and **6d** were further evaluated as drug candidates using several *in vitro* assays (Table 3). In the parallel artificial membrane permeation assay (PAMPA) to predict oral bioavailability, these compounds are ranked in the medium permeation range. In the case of CYP450 enzymes and GPCRs inhibition assay, both compounds showed no significant binding affinity. And also low cytotoxicity was observed in various cell line tests.

In summary, 1-cyclohexyl-3-aminopyrrolidine derivatives substituted with morpholine and thiomorpholine on the 4-position of cyclohexyl group were synthesized and identified as good CCR2 antagonists. The cell-based functional activities and the additional results of *in vitro* assays of selected compounds **6b** and **6d** led us to consider them as viable lead compounds.

References and Notes

1. (a) Schwarz, M. K.; Wells, T. N. *Nat. Rev. Drug Disc.* **2002**, *1*, 347. (b) Horuk, R.; Ng, H. P. *Med. Res. Rev.* **2000**, *20*, 155.
2. (a) Libby, P. *Nature* **2002**, *420*, 868. (b) Feria, M.; Diaz-Gonzalez, F. *Expert Opin. Ther. Patents* **2006**, *16*, 49. (c) Boer, W. I. *Drug Disc. Today* **2005**, *10*, 93. (d) Das, A. M.; Brodmerkel, C. M. *Drug Disc. Today: Ther. Str.* **2006**, *3*, 381.
3. Synthetic method of compound **3**, see: PCT patent, WO 2004/050024.
4. All final compounds displayed spectral data (NMR, MS) that were consistent with the assigned structures.
5. BMS CCR2 22 is commercially available from the web site, http://www.tocris.com/dispprod.php?ItemId=208254.