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Small molecule inhibitors of the CCR2b receptor. Part 1: Discovery and optimization of homopiperazine derivatives

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Abstract—*N*,*N*′-Disubstituted homopiperazine derivatives have been discovered as CC-chemokine receptor 2b (CCR2b) inhibitors with submicromolar activity in the CCR2b binding assay. A 4-substituted benzyl group on one homopiperazine nitrogen was an important moiety for binding affinity to the CCR2b receptor. The SAR for CCR2b binding affinity correlated inversely with the σ factor of the functional group on this benzyl moiety. Introduction of hydroxy groups to appropriate positions in the 3,3-diphenylpropyl group on the other homopiperazine nitrogen increased CCR2b binding activity. The synthesis of an informer library to search for alternative substructures is also described.

Chemokines, also known as chemotactic cytokines, are a family of structurally related molecules that mainly regulate inflammatory responses such as leukocyte recruitment, cellular activation, and inflammatory mediator release. They act through interaction with chemokine receptors, which are members of the G-protein coupled receptor family.[1] Monocyte Chemoattractant Protein-1 (MCP-1) is a member of the CC chemokine subfamily and is produced by macrophages, smooth muscle cells, fibroblasts, and vascular endothelial cells.

MCP-1 causes cell migration and cell adhesion of monocytes, memory T-cells and natural killer cells, as well as mediation of histamine release by basophils.[2] In addition, high expression of MCP-1 has been observed at sites of inflammation, therefore MCP-1 is thought to play an important role in inflammatory diseases such as rheumatoid arthritis,[3] atherosclerosis,[4] glomerulonephritis,[5] and multiple sclerosis.[6] For reasons described above, many groups[7] including ours, have developed programs to discover CCR2b antagonists. Among the hit compounds found from broad screening of the Teijin small molecule library and known GPCR antagonists, homopiperazine **1** was recognized as a suitable structure for traditional optimization (Fig. 1). In this paper, the hit-to-lead studies around the initial screening hit are described.

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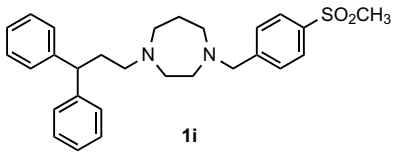
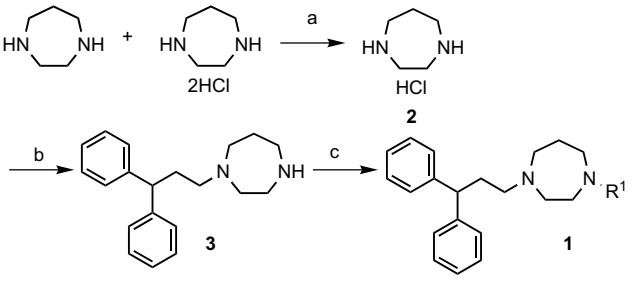


Figure 1. *N*-(3,3-Diphenylpropyl)-*N*′-(4-methylsulfonylbenzyl)homopiperazine (**1i**).



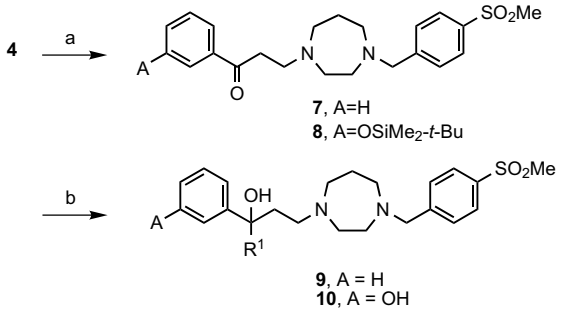
Scheme 1. Reagents and conditions: (a) EtOH; (b) $(Ph)_2CHCH_2CH_2OMs$, K_2CO_3, EtOH, heat, 93% yield; (c) R^1Cl or R^1Br, MeCN, heat, 20–70% yield.

The initial goal of the program was to optimize the homopiperazine series to potent selective CCR2b antagonists. As a separate goal, we also sought to probe for information around the lead series such that a computational model of activity could be generated and employed.[8] This paper will also discuss the identification of several informer molecules used to begin the process of lead evolution (using the pharmacophoric information from one series of molecules to identify new chemical scaffolds with similar or improved activities).

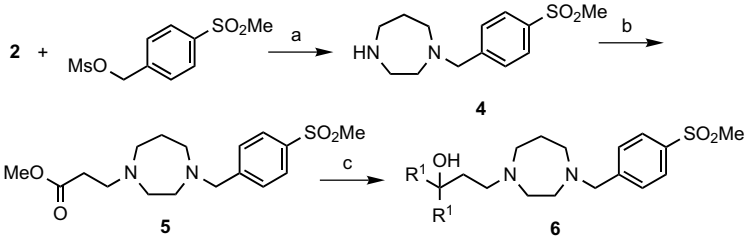
Derivatives of *N*-(3,3-diphenylpropyl)homopiperazine **1** were synthesized by mono-alkylation of homopiperazine monohydrochloride **2**, prepared in situ from equimolar amounts of free diamine and dihydrochloride salt, with 3,3-diphenylpropyl methanesulfonate, followed by a subsequent alkylation of the other nitrogen (Scheme 1).

In order to synthesize the intermediate for left-hand side modification, monobenzylation was carried out with homopiperazine monohydrochloride **2** and 4-(methylsulfonyl)benzyl methanesulfonate to obtain 1-[4-(methylsulfonyl)benzyl]homopiperazine **4** (Scheme 2). Alkylation of the second nitrogen with methyl 3-bromopropionate gave intermediate **5**, followed by a double Grignard reaction generated compounds **6** (Scheme 2). Independent substitution of each benzhydryl ring was accomplished in a similar fashion. Michael addition products **7** or **8**, formed by reaction of phenyl vinyl ketone and **4**, were prepared and immediately used without purification due to their tendency to undergo retro-Michael reactions. The addition of Grignard reagents to the freshly prepared ketones afforded the unsymmetrical compounds **9** or **10**, respectively (Scheme 3). In order to obtain **10**, desilylation with tetrabutylammonium fluoride (TBAF) in THF was carried out after mono-arylation. All the compounds described in this paper were purified using silica gel column chromatography. Purities were checked with reverse phase HPLC under isocratic condition and the structures were confirmed with ^1H NMR.

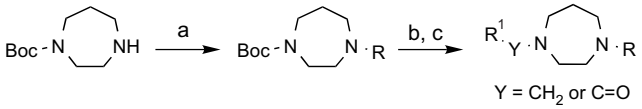
We envisioned that our computational methodology could assist in the prioritization of compounds for synthesis, either within the homopiperazine series or around alternative scaffolds. This computational methodology utilizes an ensemble of 2–4 point pharmacophores to refine the molecular space to areas where active compounds likely reside. While pharmacophore models have been employed by others to identify active compounds, our strategy differs in that only those pharmacophores capable of discriminating between active and inactive molecules become part of the model. Typically, the ensemble contains 50–100 pharmacophores. Once selected, the ensemble is then used to assist in the prioritization of compounds for synthesis. Since nearly all of the molecules in the initial dataset were closely related to **1**, locating pharmacophores that were selective for active



Scheme 3. (a) $PhCOCH{=}CH_2$ (neat) or (i) 3-TBSO–$PhCOCH{=}CH_2$, CH_2Cl_2, (ii) concentrate in vacuo; (b) (i) R^1MgBr (1 equiv), Et_2O or THF; (ii) H_3O^+, (iii) TBAF, THF for **10**, 8–62% yield for two steps.



Scheme 2. Reagents and conditions: (a) EtOH, heat, 85% yield; (b) $BrCH_2CH_2CO_2Me$, K_2CO_3, MeCN, heat, 59% yield; (c) (i) R^1MgBr (2 equiv), Et_2O or THF, (ii) H_3O^+, 10–84% yield.



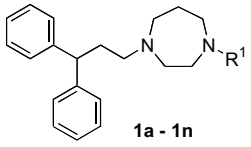
Scheme 4. Reagents and conditions: (a) RX (1.1 equiv), K_2CO_3 (5 equiv), CH_3CN, 70 °C; (b) 3 M HCl–EtOAc; (c) R^1CH_2X (1.1 equiv), K_2CO_3 (5 equiv), CH_3CN, 70 °C or R^1COOH (1.2 equiv), EDCI (1.3 equiv), HOBt (1.3 equiv), *i*-Pr_2NEt (3 equiv), DMF, rt.

molecules over inactive ones was very difficult. Therefore, to improve the quality of the early computational model, a library of homopiperazine molecules was designed to query the pharmacophoric space around the template in an overlapping fashion. Since this library was designed to procure information, the compounds from the set are called 'informer molecules'. The compounds were prepared as shown in Scheme 4. *N*-Boc-homopiperazine was heated with alkyl halide in the presence of K_2CO_3 to introduce the first R group. Removal of the Boc group followed by either a second alkylation reaction or coupling to a carboxylic acid under standard conditions, afforded the fully functionalized molecules.

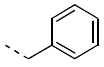
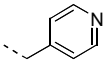
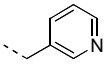
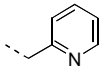
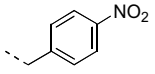
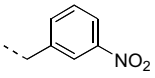
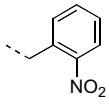
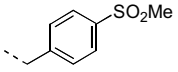
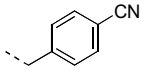
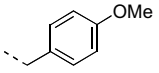
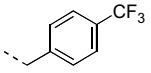
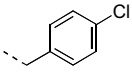
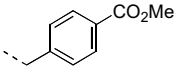
Inhibitory activity of compounds was determined against human ^{35}S-MCP-1 or ^{125}I-MCP-1 binding to THP-1 cells.[9,10] The results of selected compounds are summarized in Tables 1–5. In Table 1, the structure activity relationships around the R^1 group in **1** are shown. Compounds with simple alkyl groups (**1a**) or unsubstituted benzyl groups (**1b**) have no affinity for CCR2b. Introduction of 4-pyridylmethyl at the R^1 position (**1c**) improved activity for the receptor. A tendency of a preference for the 4-pyridyl ring was observed, and the 2- and 3-pyridyl systems exhibited reduced affinities (**1d** and **1e**). A similar preference for 4-substitution was observed for the nitrobenzyl derivatives **1f–h**. Moving the nitro group to the 2- or 3-position resulted in loss of activity. Other electron-withdrawing groups such as methylsulfonyl (**1i**) and cyano (**1j**) increased activity, while electron-donating groups like methoxy (**1k**) decreased activity. Substituents that have moderate Hammet's σ_p values in range of 0.2–0.6 also resulted in low affinity (**1l–n**).

Compound **1i**, 1-(4-methylsulfonylbenzyl)-4-(3,3-diphenylpropyl) homopiperazine (IC_{50} = 13 μM) with an EC_{50} of 5.8 μM (n = 1) in a chemotaxis assay[11] was chosen

Table 1. Binding inhibitory activity of *N*-(3,3-diphenylpropyl)homopiperazine derivatives

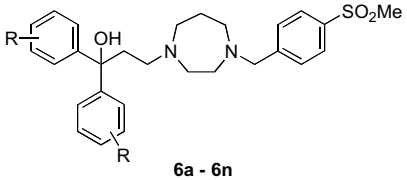


1a - 1n

Compounds	R^1	% Inhibition at 100 μM[a]	IC_{50}, μM[a]
1a		15	Nd[b]
1b		24	Nd
1c		87	11
1d		69	Nd
1e		35	Nd
1f		87	19
1g		35	Nd
1h		7	Nd
1i		91	13[c]
1j		74	43
1k		7	Nd
1l		25	Nd
1m		8	Nd
1n		1	Nd

[a] Assayed with ^{35}S-MCP-1 (n = 1 unless indicated otherwise).
[b] Not determined.
[c] SEM = 1.8 μM (n = 5).

Table 2. Effect of substituent on phenyl groups

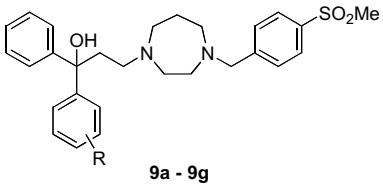


6a - 6n

Compounds	R	% Inhibition at 100 μM[a]	IC_{50} μM[a]
6a	H	104	4.1 (n = 2)
6b	4-*t*-Bu	39	Nd[b]
6c	4-Ph	0	Nd
6d	4-CF_3	38	Nd
6e	4-NMe_2	68	45
6f	4-OH	83	21
6g	3-OH	108	1.5[c]
6h	3-OMe	38	Nd
6i	2-OMe	14	Nd
6j	2-Me	0	Nd
6k	4-F	100	7.0
6l	3-F	75	34
6m	4-Cl	94	11
6n	3-Cl	28	Nd

[a] Assayed with ^{125}I-MCP-1 (n = 1, unless indicated otherwise).
[b] Not determined.
[c] SEM = 0.16 μM (n = 5).

Table 3. Derivatives of **1i** that have unsymmetrical left hand structures

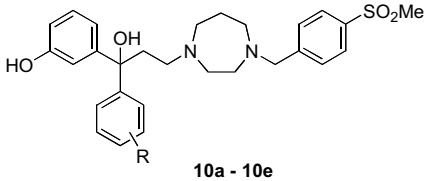


9a - 9g

Compounds	R	% Inhibition at 100 μM[a]	IC_{50}, μM[a]
9a	3-OH	105	0.71 ($n = 2$)
9b	3-CH_2OH	100	4.0
9c	3-NH_2	102	4.2
9d	3-NHMe	87	5.0
9e	3-OMe	97	7.9
9f	3-F	95	12
9g	3-Me	92	16

[a] Assayed with ^{125}I-MCP-1 ($n = 1$, unless indicated otherwise).

Table 4. Unsymmetrical derivatives of **1i** with 3-hydroxyphenyl group



10a - 10e

Compounds	R	% Inhibition at 100 μM[a]	IC_{50}, μM[a]
10a	3-F	104	2.4
10b	3-Cl	99	4.5
10c	4-F	105	1.5
10d	4-Cl	104	1.5
10e	3,5-DiF	107	9.3

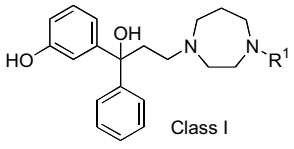
[a] Assayed with ^{125}I-MCP-1 ($n = 1$).

as the lead compound for further optimization, because of its metabolic stability and low cytotoxicity (data not shown). Therefore, the lead optimization from **1i** was carried out by structural development of the benzhydryl group. Table 2 shows the activity of symmetrical 3,3-diphenyl-3-hydroxypropyl derivatives **6**. Introduction of a hydroxy group at 3-position of the propyl chain in **1i** (**6a**) led to a 3-fold increase in binding affinity and resulted in the first compound with a low micromolar IC_{50} value. A range of activities was observed for 4-monosubstituted phenyl derivatives. Lipophilic substitution (**6b–d**) reduced activity, while dimethylamino (**6e**) and hydroxyl (**6f**) preserved inhibitory activity. Moreover, transfer of the hydroxyl group to the 3-position (**6g**) improved binding affinity, though introduction of a 3-methoxy substituent (**6h**) was not effective in augmenting activity. Compounds **6i** and **6j**, functionalized in the 2-position, showed no significant activity. The halogen-substituted compounds **6k–n** exhibited an opposite SAR as compared with the hydroxy derivatives, in that the 4-halo compounds were more active than 3-halo compounds.

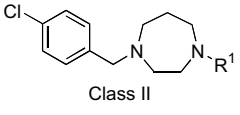
In the series of compounds **9** (Table 3) and **10** (Table 4) the two phenyl rings of the benzhydryl moiety were independently substituted.

As seen in the symmetrical derivatization (Table 2), the 3-hydroxyphenyl derivative **9a** exhibited the best activity, with an IC_{50} value of 0.71 μM (Table 3). Introduction of hydroxymethyl (**9b**), amino (**9c**), methylamino (**9d**), or methoxy (**9e**), to the 3-position of the phenyl ring decreased activity relative to **9a**, but still resulted in low μM affinities for the receptor. A decrease in activity was observed when fluoro (**9f**) or methyl (**9g**) were introduced into the same position. In comparing the halo-substituted derivatives **10** (Table 4), as seen in Table 3, 4-substitution was preferred over 3-substitution (**10a–d**). Compounds **10c** and **10d** had the same activity as **6g** but were less active than **9a**. The 3,5-difluoro

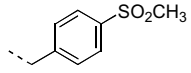
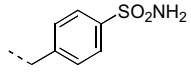
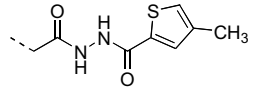
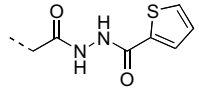
Table 5. Optimization of the benzenesulfonyl group and identification of informer molecules



Class I



Class II

R^1	Class I		Class II	
	Compound	IC_{50}, μM[a]	Compound	IC_{50}, μM[a]
	**9a**	0.71	**14**	>100
	**11**	1.5	**15**	Nd[b]
	**12**	6.4	**16**	7.4
	**13**	28	**17**	30

[a] Assayed with ^{125}I-MCP-1 ($n = 1$).
[b] Not determined.

derivative (**10e**) exhibited decreased activity as compared to the mono-fluorinated compound (**10a**). In addition, since all the compounds with an unsymmetrical benzhydryl moiety **9** and **10** were synthesized as racemic mixtures, resolution to optically active isomers should afford improvements in activity over that reported in Tables 3 and 4.

Interestingly, some of the molecules from the informer library (Table 5) exhibited activities in the range of the initial leads. It was found that in the hydrazido series, **16** and **17**, a *para*-chlorobenzyl substituent could be used as a suitable replacement for the 3,3-diphenylpropyl group. This observation did not carry over to the benzenesulfonyl series (e.g. **14**). As such, compounds **16** (EC_{50} of 9.5 μM ($n = 1$) in a chemotaxis assay) and **17** constituted a new series of MCP-1R inhibitors.

In conclusion, within the *N*-(3,3-diphenylpropyl)-*N*′-(benzyl)homopiperazine series, electron-withdrawing groups in the 4-position of the benzyl moiety such as the methylsulfonyl and the nitro substituents are favored, while electron-donating groups decrease activity. Furthermore, introduction of a hydroxyl group to the methyne carbon of the benzhydryl group as well as 3-position of one phenyl ring increased the activity of the compounds.

We also synthesized a series of informer molecules from which a new series of CCR2b inhibitors were identified (**16**, **17**). These results provided the information used to initiate the lead evolution program, which will be the subject of our next letter.

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10. Recombinant baculovirus carrying the human MCP-1 gene was constructed such that the protein could be expressed and subsequently labeled (Amersham). The THP-1 cells suspended in 50 μL assay buffer (RPMI-1640 containing 0.1% BSA and 25 mM HEPES, pH 7.4, 1×10^7 cells/mL) were treated with compound solution (25 μL) and labeled ligand (25 μL, 1 mCi/mL). After incubation and washing, the radioactivity retained by the cells was measured by scintillation counting. Dose curves of each compound with 4–6 concentration points in triplicate were generated to determine IC_{50} values.
11. THP-1 cells suspended in assay buffer (RPMI-1640 containing 10% FCS) were placed in the upper chamber (200 μL) of a 96-well micro-chemotaxis chamber™, and MCP-1 in a same solution at a final concentration of 20 ng/mL was placed in the lower chamber, with polycarbonate filter placed between the two chambers. After incubation at 37 °C for 2 h in 5% CO_2, the filter was removed, and the cells, which had migrated to the underside of the filter were fixed, stained using Diff Quick and then quantitated using a plate reader at a wavelength of 550 nm to determine the index of cell migration as a mean of three cells. Test compounds were placed in the upper and lower chambers along with THP-1 and MCP-1, respectively. Dose curves of each compound with 6–8 concentration points in triplicate were generated to determine EC_{50} values.