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Small molecule antagonists of the CC chemokine receptor 4 (CCR4)

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Abstract—The identification, optimization, and structure–activity relationship (SAR) of small-molecule CCR4 antagonists is described. An initial screening hit with micromolar potency was identified that was optimized to sub-micromolar binding potency by enantiomer resolution, halogenation of the naphthalene ring, and extension of the alkyl chain linker between the central piperidine ring and the terminal aryl group. An antagonist was identified that showed good cross-reactivity against the mouse receptor and inhibited CCR4-based cell recruitment in dose-dependent fashion.
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Chemokines are small secreted proteins which stimulate the directional migration of leukocytes through interactions with a specific subset of G-protein coupled receptors. Their role in leukocyte trafficking has made them attractive therapeutic targets for both infectious and inflammatory diseases.[1] CCR4 is a chemokine receptor[2] that is activated by the ligands MDC (macrophage derived chemokine, CCL22), TARC (thymus and activation-related chemokine, CCL17), and CKLF1 (chemokine-like factor 1),[3] and has been shown to regulate the migration of $CD4^+$ Th2 cells in disease settings.[4] Studies in mouse models of allergic airway inflammation using neutralizing antibodies against both TARC and MDC,[5] as well as receptor knockout studies[6], have demonstrated a role for CCR4 and its ligands in airway hyperresponsiveness. Taken together, these studies support a role for CCR4 antagonism in the treatment of allergic inflammatory conditions such as asthma and atopic dermatitis.

As part of our efforts to identify new potential therapeutics for CCR4-mediated inflammatory diseases, we and others[7] have sought to find novel small molecule antagonists of CCR4.[8] A high-throughput screen of our small molecule collection afforded the racemate **1** (Fig. 1) as a compound of interest that was selected for further follow-up.

In order to establish the initial SAR, we decided to capitalize on the modular nature of the molecule and to focus on the four major areas shown in Figure 1: variants at the sulfonamide substituent (R^1), variants at the amino acid amide (R^2), variants at the tertiary amine (R^3), and the absolute stereochemical requirements of the central amino acid (asterisk). The synthesis of **1** and its analogs is straightforward[9] and is shown in Scheme 1. Coupling of commercially available naphthalene-2-sulfonyl chloride with racemic proline-methyl ester afforded **2**, which was hydrolyzed with aqueous lithium hydroxide to afford the carboxylic acid **3**. Coupling with BOC-protected 4-amino-piperidine was achieved using EDCI to afford intermediate **4**. Deprotection with HCl/dioxane afforded **5**, which was reductively alkylated with benzaldehyde in the presence of sodium triacetoxyborohydride to afford **1**. Alkylation of the secondary amide to yield **6** was readily achieved using methyl iodide in the presence of sodium hydride and DMF.

Before embarking on a complete SAR survey of the molecule, we decided to examine the central amino acid

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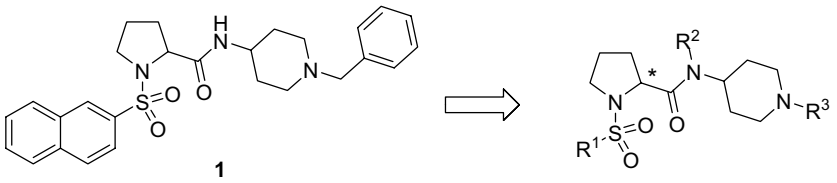
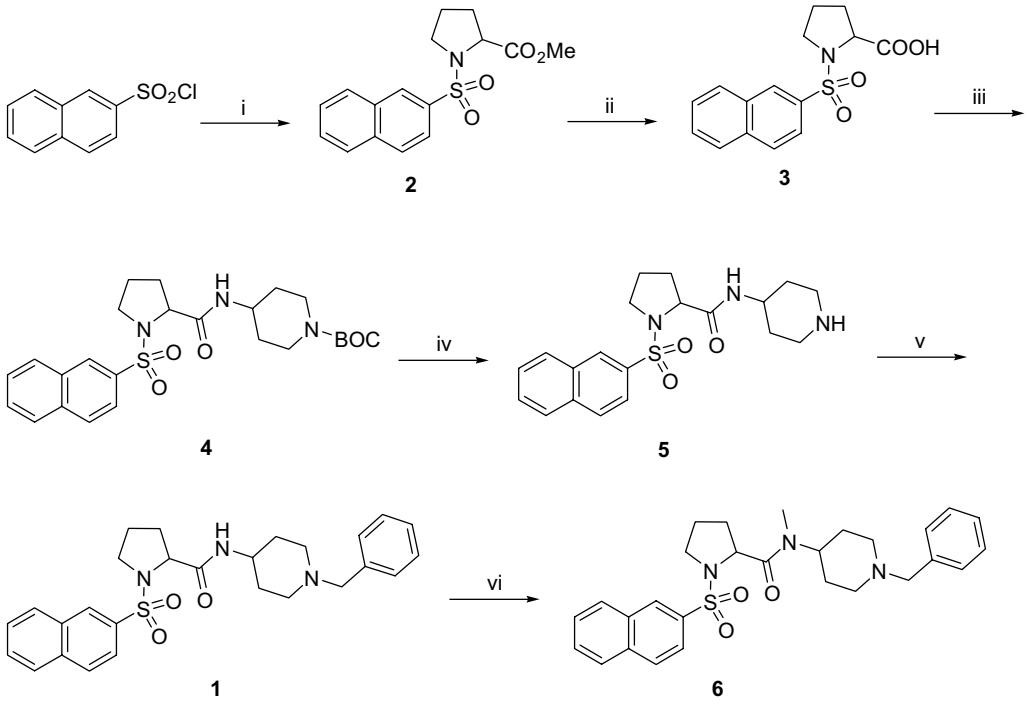


Figure 1. Structure of the initial screening hit (K_i(CCR4) = 7.6 μM) and the initial strategy for establishing SAR.



Scheme 1. Reagents and conditions: (i) (+/−)-proline-methyl ester, TEA, DCM (94%); (ii) LiOH, THF/H_2O (97%); (iii) *tert*-butyl 4-amino-piperidine-1-carboxylate, EDCI, DCM (83%); (iv) 4 N HCl, dioxane (98%); (v) benzaldehyde, $Na(AcO)_3BH$, DCM (89%); (vi) methyl iodide, NaH, DMF (63%).

linker by preparing several analogs. Substitution of a variety of amino acids in place of proline, including *N*-methyl-alanine, resulted in a complete loss of activity (data not shown) leading us to conclude that conformational rigidity was required at this position. We next sought to establish the stereochemical requirements for the proline linker and turned our attention to preparing the enantiomers **7** and **8** (Fig. 2) from D- and L-proline-methyl ester, respectively. We identified the *S*-enantiomer **8** as the more active enantiomer, and this compound was used as a starting point for further optimization.

An initial SAR survey of **8** was undertaken in order to determine the best path for optimization (Table 1). We were quickly able to establish a requirement for the sulfonamide moiety as a substantial loss in potency was witnessed with the carboxamide **9**, the amine **10**, and the urea **11**. The requirement for a bulky aromatic group at R^1 also became apparent as the benzenesulfonamide **12** and the isopropyl sulfonamide **14** lost activity. Insertion of a methylene spacer, as in **13**, was also detrimental. Interestingly, positioning of the sulfonamide moiety at the 1-position of the naphthalene ring, as exemplified by **18**, also resulted in a loss of potency. Although sub-

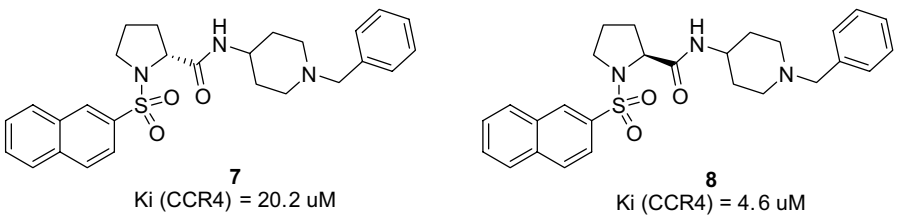
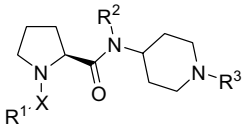


Figure 2. Identification of the more active enantiomer **8**.

Table 1. Initial tepresentative SAR



Compound	X	R^1	R^2	R^3	CCR4 FLIPR K_i (μM)
8	SO_2	2-Naphthyl	H	CH_2–Ph	4.6[a]
9	CO	2-Naphthyl	H	CH_2–Ph	>29[b]
10	CH_2	2-Naphthyl	H	CH_2–Ph	25[b]
11	(CO)NH	4-*tert*-Butylphenyl	H	CH_2–Ph	>20[a]
12	SO_2	Phenyl	H	CH_2–Ph	>29[b]
13	SO_2	Benzyl	H	CH_2–Ph	15[b]
14	SO_2	Isopropyl	H	CH_2–Ph	>29[b]
15	SO_2	4-Isopropylphenyl	H	CH_2–Ph	10[a]
16	SO_2	2-Naphthyl	H	H	>33[b]
17	SO_2	2-Naphthyl	H	Isobutyl	10[b]
18	SO_2	1-Naphthyl	Me	CH_2–Ph	26[b]
19	SO_2	2-Naphthyl	H	CH_2-2-pyridyl	>10[a]
20	SO_2	2-Naphthyl	H	CH_2-3-pyridyl	>10[a]
21	SO_2	2-Naphthyl	Me	CH_2–Ph	1.3[a]

[a] Value determined[10] using TARC as a ligand.
[b] Value determined[10] using MDC as a ligand.

stitution of an isopropyl group on the phenyl ring, as exemplified by **15**, conveyed modest potency, a 2-naphthyl ring at R^1 proved consistently more potent and we proceeded to optimize the remainder of the molecule with this substituent comprising an essential component of the pharmacophore.

We next turned our attention to the central and right-hand portions of the molecule. Our most consistent jump in activity was realized upon methylation at R^2; comparison of the methylated derivative **21** with its hydrogen-substituted counterpart **8** revealed a modest increase in activity. A cursory examination of the piperidine substituents revealed a requirement for alkylation at R^3. The secondary amine **16** was inactive, while bulkier alkyl substituents, as in **17**, restored some activity. Heteroaryl substitutions, as in **19** and **20**, were not tolerated. We returned ultimately to the benzyl substituent in **21**, which showed a potency of 1.3 μM and was selected for further optimization.

With the lead compound **21** in hand, we sought to improve the potency by optimizing the substituents at R^1, R^2, R^3, and R^4 as shown in Table 2.

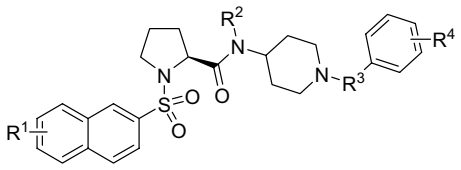
A length scan at R^3 revealed an improvement in potency in the 2-carbon linker as exemplified by **22**. The 3-carbon linker in **23** offered no improvement in potency and the additional rotatable bond and molecular weight were deemed unnecessary. The acyl derivative **24** and the sulfonamide **25**, prepared from the corresponding amine and acid chlorides, were inactive. We turned next to the amide substituent at R^2, mindful of the improved potency conveyed by N-methylation. Synthesis of the *N*-ethyl derivative **26**, however, led to a significant loss of potency, and therefore further methylene homologs were not pursued. Substitution with polar groups (**27**), both basic (**29**) and acidic (**28**), resulted in a complete loss of activity leading us to conclude that a methyl group was optimal at that position.

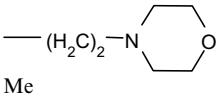
Armed with an understanding of the SAR surrounding the central portions of the scaffold, we next sought to optimize the substituents at the termini. The naphthalene ring proved least accessible to diversification owing to the limited number of synthetically accessible 5-substituted naphthalene-2-sulfonic acids. Nevertheless, the fluoro-substituted analog **30** could be accessed by thermal decomposition of the diazonium ion in the presence of BF_3 etherate,[11] but showed no improvement in potency. Polar group substitutions, as in **31** and **32**, were not tolerated. We were gratified to see a substantial increase in potency in the chloro-substituted derivative **33**, and this functional group was maintained constant as we sought to optimize the terminal arene ring.

Our efforts to improve potency by substitution of the phenethyl ring proved frustrating. Halogen substitution at the *ortho* (**34**, **35**), *meta* (**36**) or *para* (**37**) position led to a modest decrease in potency, while electron donating substituents (**38**) and electron withdrawing substituents (**42**) had a similar effect. Polar substituents (**40**), both basic (**41**) and acidic (**39**), also led to a modest decrease in potency, thereby leaving no clear path forward for optimization. Nevertheless, the reasonable potency seen in **33** prompted us to profile this compound further. As shown in Figure 3, **33** proved nearly equipotent when assayed against murine CCR4 using murine MDC. This cross-reactivity presented an attractive opportunity to evaluate this molecule in a murine recruitment model and prompted us to profile this compound in an in vivo efficacy experiment.

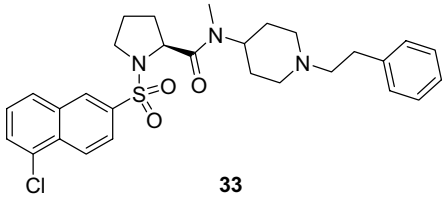
In order to evaluate the ability of CCR4 antagonists to inhibit chemotaxis in vivo, we performed an early proof of concept study utilizing a peritoneal recruitment assay.

Table 2. Optimizing the substituents at R^1, R^2, R^3, and R^4



Compound	R_1	R_2	R_3	R_4	CCR4 FLIPR K_i (μM)[a]
21	H	Me	$–CH_2–$	H	1.3
22	H	Me	$–CH_2–CH_2–$	H	0.59
23	H	Me	$–(CH_2)_3–$	H	0.62
24	5-Cl	Me	$–(CO)–CH_2–$	H	>10
25	H	Me	SO_2	H	>10
26	5-Cl	Et	$–CH_2–CH_2–$	H	2.80
27	H	$–CH_2–CO_2Et$	$–CH_2–CH_2–$	H	>10
28	H	$–CH_2–CO_2H$	$–CH_2–CH_2–$	H	>10
29	H		$–CH_2–CH_2–$	H	>10
30	5-F	Me	$–CH_2–CH_2–$	H	0.85
31	5-NH_2	Me	$–CH_2–CH_2–$	H	>10
32	5-NHAc	Me	$–CH_2–CH_2–$	H	>10
33	5-Cl	Me	$–CH_2–CH_2–$	H	0.10
34	5-Cl	Me	$–CH_2–CH_2–$	2-F	0.26
35	5-Cl	Me	$–CH_2–CH_2–$	2-Cl	0.46
36	5-Cl	Me	$–CH_2–CH_2–$	3-Cl	0.40
37	5-Cl	Me	$–CH_2–CH_2–$	4-Cl	0.82
38	5-Cl	Me	$–CH_2–CH_2–$	3-MeO	0.47
39	5-Cl	Me	$–CH_2–CH_2–$	2-COOH	0.53
40	5-Cl	Me	$–CH_2–CH_2–$	2-$CONH_2$	0.24
41	5-Cl	Me	$–CH_2–CH_2–$	2-NH_2	0.28
42	5-Cl	Me	$–CH_2–CH_2–$	2-CF_3	0.42

[a] Values determined[10] using TARC as a ligand.



33

K_i (hTARC/FLIPR) = 100 nM
K_i (hMDC/FLIPR) = 500 nM
K_i (mMDC/FLIPR) = 150 nM

Figure 3. Structure of **33**, a potent CCR4 antagonist used in a proof of concept study.

Briefly, compound **33** was administered sub-cutaneously to BALB-C mice (6 animals/cohort). After 30 min, mMDC (1 μg) was administered intraperitoneally; after 90 min, cells were withdrawn from the peritoneum, sorted, and counted.

As shown in Figure 4, we were pleased to see that **33** inhibited the recruitment of cells to the peritoneum in dose-dependent fashion, with a sub-cutaneous dose of 5 mpk inhibiting recruitment nearly 90%. Based on our PK analysis (data not shown), this dose delivered a maximum exposure of 600 nM with exposure exceeding the IC_{50} throughout the course of the experiment.

In summary, we have identified and optimized a series of naphthalene-sulfonamides that are functional antagonists of CCR4. In light of the ability of compound **33** to block recruitment of cells in response to mMDC, it will be interesting to evaluate these compounds in animal models of $Th2^+$ disease. These results will be reported in due course.

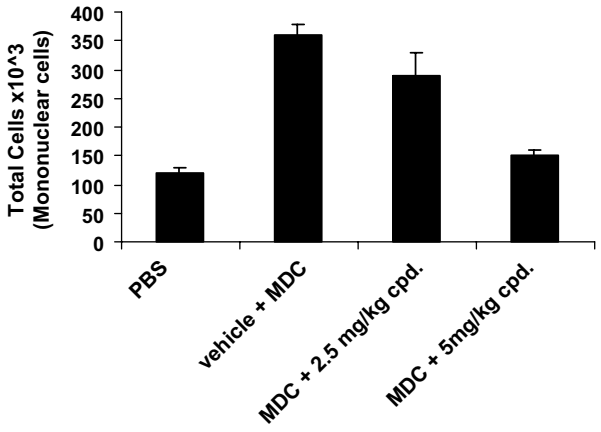


Figure 4. Dose response of **33** to peritoneal recruitment by mMDC.

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9. All compounds were characterized by LC/MS and ^{1}H NMR. Chiral HPLC analysis of **7** and **8** revealed an enantiomeric excess (ee) of >98%.
10. This screen is based on the use of a stable Chinese hamster ovary cell line that overexpresses recombinant CCR4 receptor and Gα16 protein, whose activation causes intracellular calcium mobilization. Agonist-promoted increases in intracellular calcium were detected with a fluorescence imaging plate reader (FLIPR) using a calcium-sensitive dye. The ability of compounds to antagonize binding of MDC or TARC to cell surface CCR4 is reflected in the reduction of fluorescence signal relative to positive controls. Final assay volume is 60 μL. Final concentrations of key components are as follows: MDC: 15 nM or TARC: 10 nM; Test compound: variable; dimethylsulfoxide 1.0% (v/v), bovine serum albumin: 1.0 mg/mL; HEPES: 20 mM; probenecid: 2.5 mM; 10,000 cells per well. A counterscreen against UTP was performed in parallel in order to screen out cytotoxic molecules. Good correlations between K_i values were observed when comparing assays utilizing TARC and MDC as ligands.
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