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Optimization of CCR4 antagonists: Side-chain exploration

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Abstract—The design, synthesis, and activity of novel and selective small molecule antagonists of the CC chemokine receptor-4 (CCR4) are presented. Compound **8c** was efficacious in a murine allergic inflammation model (ED_{50} 30 mg/kg).

Chemokines and their receptors act as a communication and signaling network between cells, organs, and regions of the body to facilitate immunological responses.[1] Chemokine receptor-4 (CCR4) together with its ligands from the CC chemokine family, macrophage derived chemokine (MDC; CCL22) and thymus and activation regulated chemokine (TARC; CCL17), are responsible for recruitment, homing, and education of activated leukocytes (mainly $CD4^+$ Th2 lymphocytes).[2] Recently, CCR4 and its ligands have attracted significant attention due to their involvement in mediating various allergic inflammatory conditions such as asthma, acute dermatitis, etc.[3] Supported by studies using monoclonal antibodies for the CCR4 receptor and its ligand TARC in OVA-induced murine asthma models,[4] we[5] and others[6] have targeted antagonism of the CCR4 receptor as a mechanism of inhibiting recruitment of activated leukocytes to the lungs, thus supporting CCR4 antagonism as a potential treatment for diseases such as asthma and atopic dermatitis.

Recently, we reported[5] the identification of a pyrimidine analog (**1** in Fig. 1) as a non-cytotoxic and moderately potent antagonist of CCR4 (MDC binding). We herein report further SAR exploration of the side chain that led to the identification of a potent CCR4 antagonist with in vivo activity.

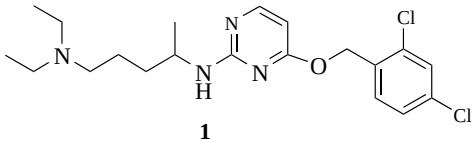

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Figure 1. CCR4 IC_{50} 1.5 μM.

We first embarked on a systematic study of the diamine side chain of compound (**1**). A small array of compounds (**4a**–**g**) containing different diamine linkers and terminal R^1 groups was synthesized as shown in Scheme 1. Intermediates (**I**–**IV**) where R^1 = *O*-*t*-butyl were synthesized by coupling of pyrimidine derivative **3**[5] with the requisite mono-Boc-containing diamines. N-Boc deprotection of intermediates (**I**–**IV**) and subsequent amide bond formation with acids bearing basic R^1 groups [7] provided compounds (**4a**–**g**). Amongst the different linkers and terminal R^1 groups, piperazine and 1-methylnipecotate; respectively; were preferred, (compounds **4c** and **4d**, Table 1) over other nitrogen containing aromatic heterocycles or linkers (compounds **4e**–**g**).

Next we examined the effect of the linking atom (N vs O) and regiochemistry of attachment to the central core. The compounds were prepared from the previously reported intermediates (**5a** and **5b**)[6] as shown in Scheme 2.[7] A 2-fold enhancement of activity by changing the linking atom from oxygen to nitrogen atom was observed (compound **7a**; Table 2).[8] This trend was the same as that observed in a previously reported series.[6] The regioisomer **7b** was found to be 2-fold more active than **7a**.

Encouraged by these findings, we embarked upon further optimization of the terminal amino moiety.

Keywords: Chemokine receptor CCR4; Antagonist; MDC; In vivo active; Asthma.
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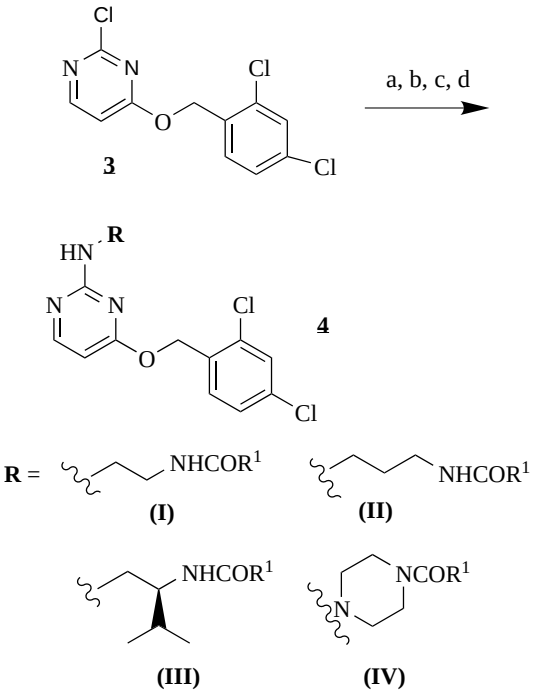
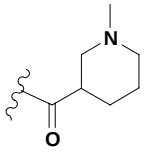
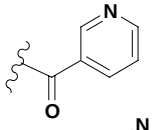
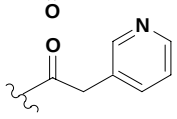
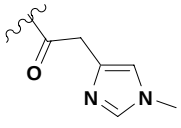


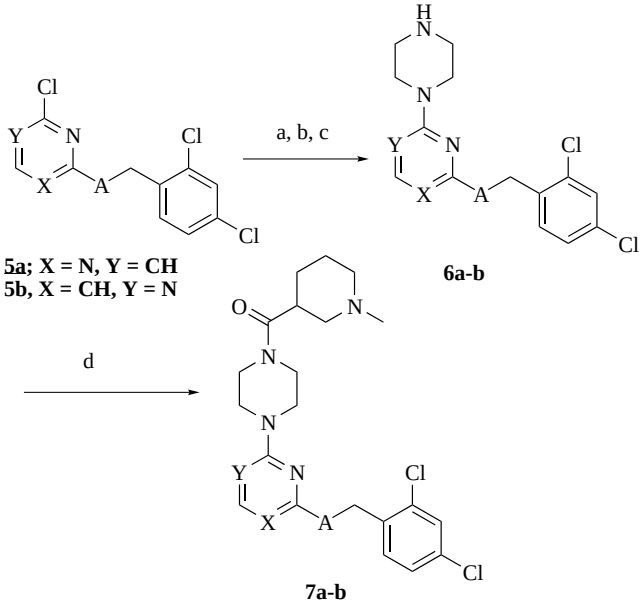
Scheme 1. Reagents and conditions: (a) **I–IV** where R_1 = –OtBu, *N*-methyl-pyrrolidone, 60 °C, 70–82%; (b) TFA-CH_2Cl_2 (1:3), 90–95%; (c) SCX purification, 93–98%; (d) R_1CO_2H, 1,3-di-isopropylcarbodiimide, 1-hydroxy-7-azabenzotriazole, DMF–CH_2Cl_2 (1:1), 60–70%.

Table 1. SAR for linker

Compound	Linker group	R^1	CCR4 IC_{50} (μM)[8]
4a	**I**		>10
4b	**II**	Same	>10
4c	**III**	Same	5.6
4d	**IV**	Same	1.2
4e	**IV**		>10
4f	**IV**		>10
4g	**IV**		>10

The analogs were prepared from the intermediate **6b** and Boc-protected cyclic amino acids followed by the Boc group deprotection as shown (Scheme 3).[7]

As shown in Table 3, α-amino acids at the N terminus were found to be preferred over either β or γ-amino acid



Scheme 2. Reagents and conditions: (a) *N*-Boc-piperazine, *N*-methyl-pyrrolidine, 90 °C, 82–85%; (b) TFA–CH_2Cl_2 (1:3), 98%; (c) SCX purification, 92%; (d) 1-methylnipecotoic acid,1,3-di-isopropylcarbodiimide,1-hydroxy-7-azabenzotriazole, DMF–CH_2Cl_2 (1:1), 89%.

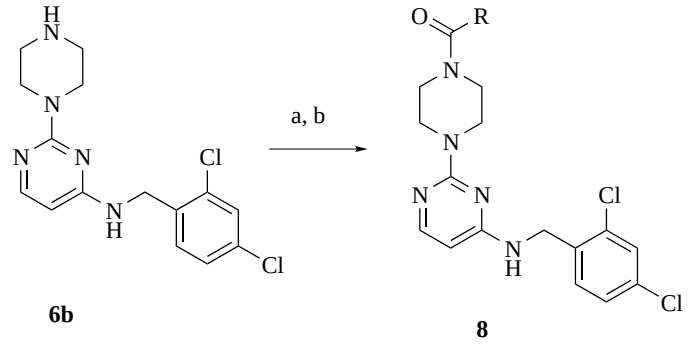
Table 2. SAR for ring atoms

Compound	A	X	Y	CCR4 IC_{50} (μM)[8]
4d	O	N	CH	1.2
7a	NH	CH	N	0.7
7b	NH	N	CH	0.3

analogs. Within the subset made from α-amino acids, the *R*-enantiomer was about 15-fold more active than the corresponding *S*-enantiomer. The same trend was observed in general across different cyclic amino acids but there was no significant difference in the activity for five- versus six-membered rings (i.e., proline **8d** vs homoproline **8c**).

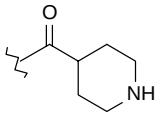
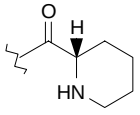
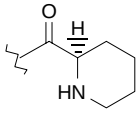
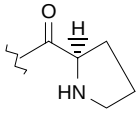
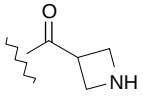
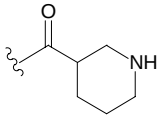
Compound **8c** was not only active in the in vitro CCR4 binding assay, but it also blocked chemotaxis (IC_{50} 0.38 μM) and Ca^{2+} mobilization (IC_{50} 0.01 μM).[8,9] It was >1000-fold selective against related chemokine receptors (CCR3, CCR2, and CXCR3) and GPCRs (5-HT1A, 5-HT6, and 5-HT7).[8]

Compound **8c** was administered sub-cutaneously twice a day at 10, 30, and 100 mg/kg dose to mice previously challenged with ovalbumin (Fig. 2).[10] Dexamethasone administered orally, 5 mg/kg was used as control to assess the relative efficacy. Compound **8c** reduced the recruitment of eosinophils with an ED_{50} of 30 mg/kg. Additionally at 100 mg/kg dose, **8c** was as efficacious as dexamethasone 5 mg/kg in reducing eosinophilic infiltration into mouse BAL. These results clearly demonstrate the proof of principle of using a small molecule CCR4 antagonist as a potential treatment for asthmatic disease.



Scheme 3. Reagents: (a) R_1CO_2H, 1,3-di-isopropylcarbodiimide,1-hydroxy-7-azabenzotriazole, DMF–CH_2Cl_2 (1:1); (b) TFA–CH_2Cl_2 (1:3), 65–75%.

Table 3. SAR for terminal amine

Compound	R	CCR4 IC_{50} (μM)[8]
8a		>10
8b		1
8c		0.05
8d		0.06
8e		>10
8f		0.8

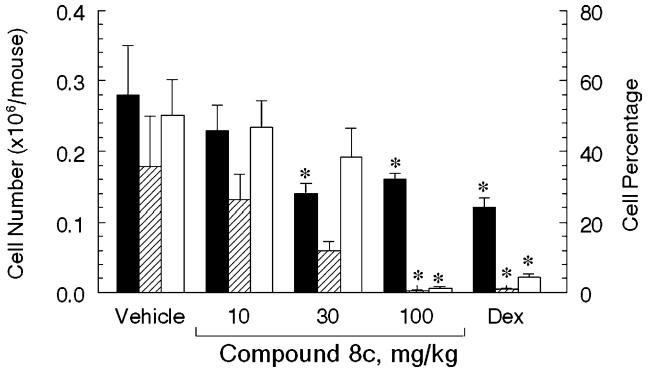
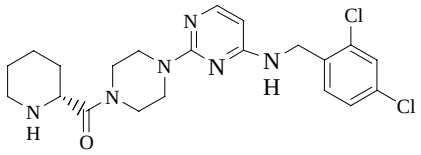


Figure 2. Dose-dependent inhibition by compound **8c** of eosinophil infiltration into allergic lung airways. Total leukocytes (solid bars), total eosinophils (hatched bars), and percent eosinophils (open bars) in bronchoalveolar lavage (BAL) fluid were determined as described.[10] Data represent means ± SEM of seven mice treatment group. *$p < .05$ versus vehicle group, Student's t test.



8c
CCR4 IC_{50} 0.05 μM
Cytotoxicity IC_{50} > 100 μM
Chemotaxis IC_{50} 0.38μM
Ca^{2+} mobilization 0.01μM

In summary, we have identified a potent and selective CCR4 antagonist (compound **8c**) that showed in vivo efficacy in a murine allergic lung inflammation model. Further studies pertaining to optimization of this compound will be reported in due course.

References and notes

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7. All compounds were characterized by LC–MS and NMR analysis. In addition, the yields were based on the weight of pure product unless mentioned otherwise.
8. In vitro (MDC binding, chemotaxis, and cytoxicity) assays were performed using conditions as described in Ref 5. Substitution of TARC in the binding assay for MDC gave IC_{50} values within 2-fold of the MDC based values.
9. Calcium mobilization—log-sphase CEMS529 cells were washed in loading buffer that contained Hanks' balanced salt solution (GIBCO #14025-076) supplemented with 10 mM Hepes (pH 7.2) and 0.1% bovine serum albumin (Sigma #A7284), and re-suspended in loading buffer at 2×106 cells/ml. Fluo-4 (Molecular Probes #F-14201) was added to the cells at 4 μg/ml and the cells were incubated at 37 °C for 45 min. Following incubation, the cells were centrifuged, the excess dye was removed, the cells were washed once with loading buffer, and the cells are re-suspended in loading buffer at 2×106 cells/ml. 1×105 cells were added to each well of a 96-well BIOCOAT plate (#36-6640) and the cells adhered to the plate by centrifugation. The cells were then pre-incubated with selected compounds for 20 min at room temperature before the calcium mobilization in response to an empirically determined amount of MDC ligand was measured by FLIPR.
10. OVA lung inflammation in mice: BALB\c female mice, 6–8 weeks of age (Harlan, Indianapolis), were immunized intraperitoneally with 100 μg of ovalbumin (OVA; Sigma) in alum adjuvant (Pierce) and similarly boosted 10 days later. Ten days after the booster, the mice were challenged intranasally with 100 μg OVA in 50 μl pyrogen-free saline. Seventy-two hours after challenge, mice were killed by barbiturate overdose and lungs were lavaged via a tracheostomy with 1 ml ice-cold Hanks' balanced salt solution (Ca^{2+}- and Mg^{2+}-free) containing 10% fetal bovine serum. Total recovered leukocytes were enumerated by electronic cell counter (Scharfe System, Reutingen, Germany). Cytocentrifuge smears (Shandon, Pittsburg) were stained with Wright–Giemsa stain and 200 cells per sample were classified by microscopic differential.