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Core exploration in optimization of chemokine receptor CCR4 antagonists

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Abstract—The design, synthesis, and SAR studies of 'core' variations led to identification of novel, selective, and potent small molecule antagonist (**22**) of the CC chemokine receptor-4 (CCR4) with improved in vitro activity and liability profile. Compound **22** was efficacious in a murine allergic inflammation model ($ED_{50} \sim 10$ mg/kg).

The chemokine receptor CCR4 works together with its ligands from the CC chemokine family, macrophage-derived chemokine (MDC; CCL22), and thymus and activation regulated chemokine (TARC; CCL17), to promote recruitment, homing, and education of activated leukocytes (mainly $CD4^+$ Th2 lymphocytes).[1] In addition, a new ligand for CCR4 has recently been described called CKLF1 (chemokine-like factor 1)[2] whose role in immuno-modulation is unclear. Studies[3] using monoclonal antibodies for the CCR4 receptor and its ligand TARC have demonstrated efficacy in OVA-induced murine asthma models.[4] We[5] and others[6] have demonstrated targeted antagonism of the CCR4 receptor as a mechanism of inhibiting recruitment of activated leukocytes. These studies support the mechanism of CCR4 antagonism as a potential therapeutic treatment for diseases such as asthma and atopic dermatitis.

Recently, we reported[5] the identification of pyrimidine analogs (**1** and **2** in Fig. 1) as potent antagonists of CCR4, wherein we optimized the 'wing' or sidechain portions. We envisaged that the basic pharmacophore for CCR4 binding of such compounds consisted of two 'wings' attached to a central 'core' in a defined configuration (Fig. 2). Such an arrangement presented the

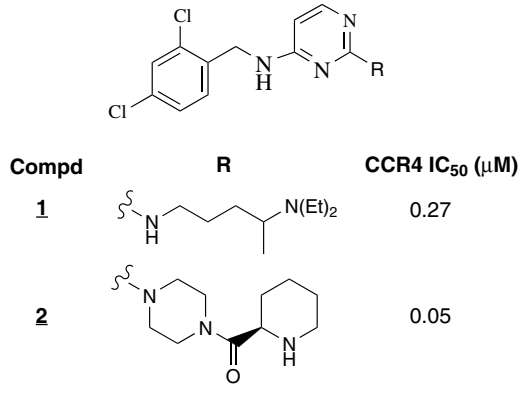

Figure 1.

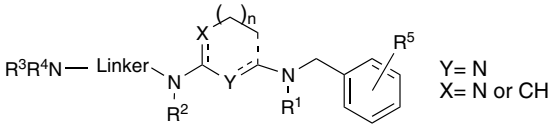

Figure 2.

'wing' groups to the desired residues in the receptor protein for favorable interactions.

We herein report our efforts to explore and further optimize the 'core' that led to the identification of a potent CCR4 antagonist **22** with an improved liability profile and in vivo activity.

Keywords: Chemokine receptor CCR4; Antagonist; MDC; TARC; Asthma; Atopic dermatitis.
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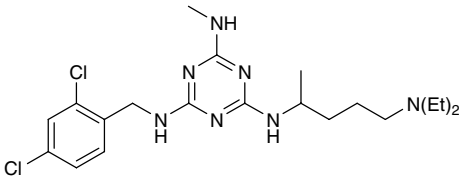
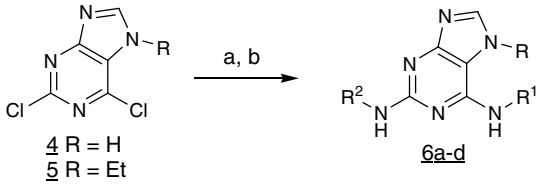


3 CCR4 IC_{50} (μM)[12] 3.6 μM

Figure 3.



4 R = H
5 R = Et

6a-d

Scheme 1. Reagents and conditions: (a) R^1NH_2, 1,2-dichloroethane, rt, 75–80%; (b) R^2NH_2, NMP, 85 °C, 60–65%.

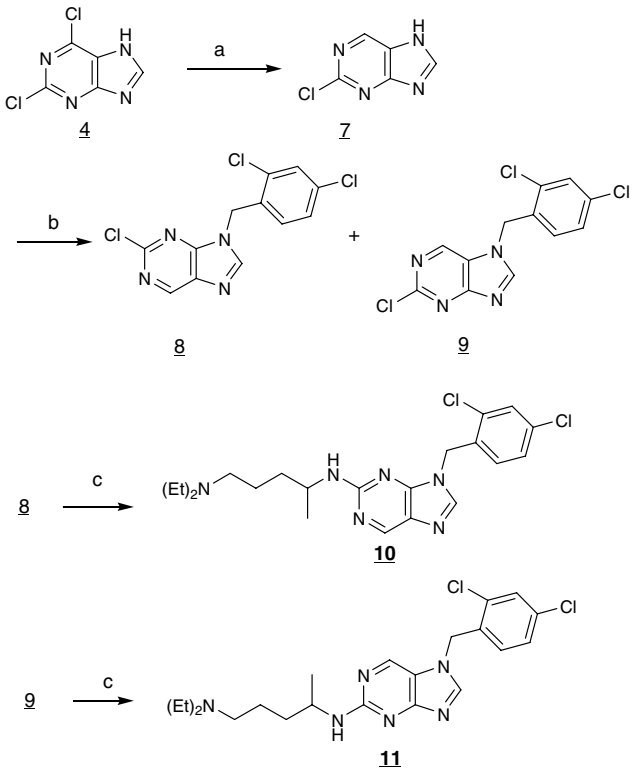
We first embarked on a systematic study of the 'core' while retaining the 2,4-dichlorobenzyl and the diamine sidechain present in **1**. Our attempt to convert the pyrimidine to a triazine core (**3**) led to ~15-fold loss of activity (see Fig. 3).

We then surveyed the purine nucleus (*N*-7 alkylated and unsubstituted) while maintaining the desired 1,3-directionality. These analogs could be readily accessed from reported intermediates[7] (**4**–**5**) through sequential displacement of the chlorine atoms (Scheme 1).[8]

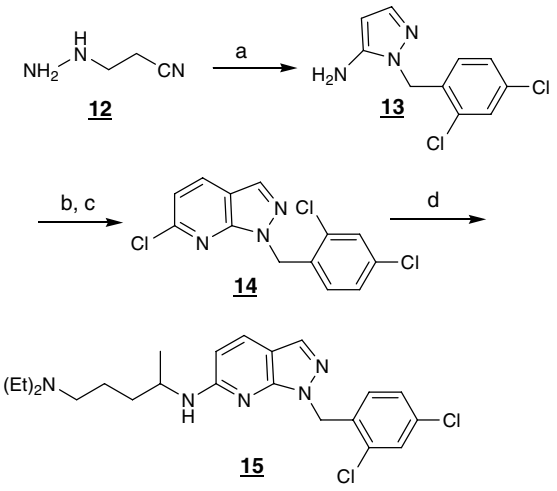
In either regioisomeric 'wing' arrangements, *N*-7 alkylated analogs (**6a** and **b**) were 4- to 5-fold less active than **1**. (Table 1) Also, *N*-7 unsubstituted analogs (**6c**–**d**) were about 2-fold less active than the corresponding *N*-7 alkylated analogs.

Alternatively, we maintained the 1,3-disposition of the sidechains in the purine nucleus by directly attaching the benzyl group to the *N*-7 atom. The synthesis of this analog was carried out as shown in Scheme 2.[8,9] During the synthesis, about 25% of regioisomer **9** was also obtained, which was used to prepare analog **11** having a 1,4-disposition. In addition, to further understand the effect of relocating the nitrogen atom, we prepared the corresponding pyrazolo[3,4-*d*]pyridine analog using the approach depicted in Scheme 3.[8,10]

These analogs showed a 3-fold reduction in activity as compared to **1** (Table 2). Interestingly, there was no difference in the activity when the sidechains were disposed in *meta* (1,3-) (**10**) or *para* (1,4-) (**11**) configurations. Pyrazolo[3,4-*d*]pyridine analog (**15**) also showed a similar level of activity as **1**.



Scheme 2. Reagents and conditions: (a) Ref. 8, 70%; (b) 2,4-dichlorobenzyl chloride, K_2CO_3, NMP, rt, 60–65%; (c) H_2N–(CH)Me–$(CH_2)_3N(Et)_2$, NMP, 90 °C, 55–60%.



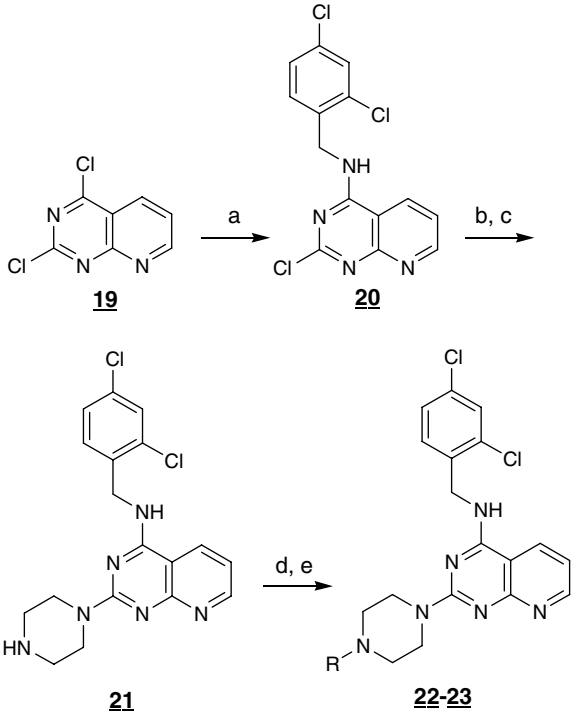
Scheme 3. Reagents and conditions: (a) 2,4-dichlorobenzaldehyde, H_2, Pd/C; 70%; (b) ethyl propiolate, acetic acid, 110 °C, 55%; (c) $POCl_3$, reflux, 80%; (d) H_2N–(CH)Me–$(CH_2)_3N(Et)_2$, NMP, 130 °C, 55–60%.

Table 1. SAR for purines

Compound	R	R^1	R^2	CCR4 IC_{50} (μM)[12]
6a	H	–(CH)Me–$(CH_2)_3N(Et)_2$	2,4-Di-Cl-benzylamine	2.1
6b	H	2,4-Di-Cl-benzylamine	–(CH)Me–$(CH_2)_3N(Et)_2$	4.9
6c	Et	2,4-Di-Cl-benzylamine	–(CH)Me–$(CH_2)_3N(Et)_2$	1.1
6d	Et	–(CH)Me–$(CH_2)_3N(Et)_2$	2,4-Di-Cl-benzylamine	2.6

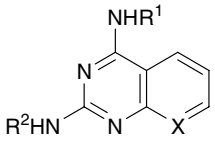
Table 2. SAR for *N*-7 benzyl analogs

Compound	CCR4 IC_{50} (μM)[12]
10	0.82
11	0.83
15	0.28



Scheme 4. Reagents and conditions: (a) 2,4-dichlorobenzylamine, 1,2-dichloroethane, Hunig's base, rt, 85%; (b) Boc-piperazine, NMP, 90 °C, 75%; (c) TFA–CH_2Cl_2 (1:1), rt, 90%; (d) *N*-Boc-*R*-homoproline or *N*-Boc-*R*-proline, DIC, HOAt, DMF, rt, 90%; (e) TFA–CH_2Cl_2 (1:4), rt, 90%.

Next, we explored the quinoline nucleus in both regioisomeric forms. Syntheses of these analogs (**16**–**18**, Table 3) were achieved using general procedures as reported in the literature.[8,11] Regioisomer **17** was 2-fold less active than **1**, whereas the other regioisomer **16** was 8- to 9-fold less active. Further, introduction of a nitrogen atom in the quinoline core afforded pyrido[2,3-*d*]pyrimidine **18**, which was 2-fold more active than **1**.



16-18

Table 3. SAR for quinolines

Compound	X	R^1	R^2	CCR4 IC_{50} (μM)[12]
16	CH	–(CH)Me–$(CH_2)_3N(Et)_2$	2,4-Di-Cl-benzylamine	1.91
17	CH	2,4-Di-Cl-benzylamine	–(CH)Me–$(CH_2)_3N(Et)_2$	0.5
18	N	2,4-Di-Cl-benzylamine	–(CH)Me–$(CH_2)_3N(Et)_2$	0.14

Table 4. SAR for terminal amine

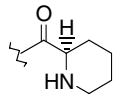
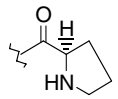
Compound	R	CCR4 IC_{50} (μM)[12]
22		0.02
23		0.08

Table 5. In vitro profile of **1** and **22**

Assay	Compound **1**	Compound **22**
CCR4 IC_{50} (μM)[12]	0.28	0.02
Chemotaxis inh. IC_{50} (μM)	5	0.007
Inh. of Ca^{2+} mobilization IC_{50} (μM)	0.8	0.003
Human microsome stability (nmol/min/mg)	0.10	0.003
HERG IC_{50} (μM)	0.3	>30
CYP IC_{50} (μM)	All >1	All >8
HHA IC_{50} (μM)	>40	>40
SOS Chromotest	Negative	Negative

Encouraged by these findings, we introduced the optimized wings[5b] from **2** onto this core. Synthesis of these analogs was carried out using the route in Scheme 4.[8] The resulting pyrido[2,3-*d*]pyrimidine **22** was 2-fold more active in the CCR4 binding assay compared to the pyrimidine analog **2** (Table 4). A similar trend in the activity as seen in the case of **2** through variation of the terminal amino acid sidechain was also observed (**22** vs. **23**) for CCR4 binding.[5b] Compound **22** also blocked MDC-mediated chemotaxis (IC_{50} 0.007 μM) and Ca^{2+} mobilization (IC_{50} 0.003 μM).[12] It was >500-fold selective against related chemokine receptors (CCR3, CCR2, and CXCR3) and GPCRs (5-HT1A, 5-HT6, and 5-HT7).

As evident (from Table 5), compound **22** showed improved in vitro activity, reduced potential to cause drug–drug interaction (IC_{50} > 8 μM for all CYP isozymes), and likely lower clearance (human microsome stability assay rate; 0.003 nmol/min/mg) as compared to **1**. Compound **22** also displayed lower possibility to cause cardiac, hepatic, and carcinogenicity liabilities based upon its in vitro activities in the hERG functional (patch clamp) (IC_{50} > 30 μM), immortalized hepatocyte (HHA) (IC_{50} > 40 μM), and SOS chromotest (+S9/−S9; negative) assays, respectively.

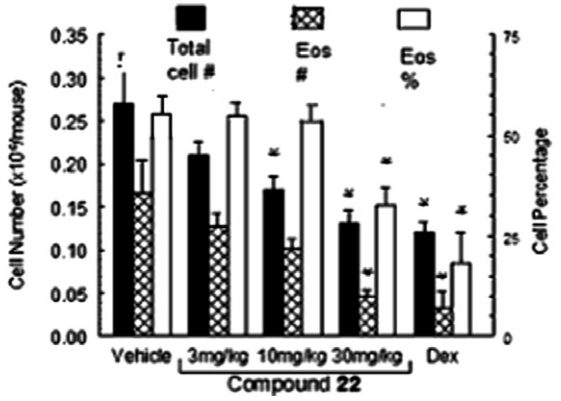


Figure 4. Dose-dependent inhibition by compound **22** of eosinophil infiltration into allergic lung airways. Total leukocytes (solid bars), total eosinophils (hatched bars), and percent eosinophils (open bars) in bronchoalveolar lavage (BAL) fluid were determined as described.[13] Data represent means ± SEM of seven to eight mice per treatment group. $^{*}p < 0.05$ vs. Vehicle group, ANOVA with Dunnett's test.

In vivo, compound **22** was administered subcutaneously twice a day at doses of 3, 10, and 30 mg/kg to mice previously immunized with ovalbumin (Fig. 4).[13] Dexamethasone administered orally, 5 mg/kg, was used as a control to assess the relative efficacy. Compound **22** reduced the recruitment of eosinophils with an ED_{50} of ~10 mg/kg (as compared to ED_{50} of 30 mg/kg twice a day for **2**[5b]). Additionally, at the 30 mg/kg dose, **22** was as efficacious as dexamethasone in reducing eosinophilic infiltration into mouse BAL.

In summary, we have identified a potent and selective CCR4 antagonist (compound **22**) with an improved liability profile that showed in vivo efficacy in a murine allergic lung inflammation model.

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12. In vitro assays (^{125}I-MDC binding with stably expressed human CCR4 in HEK-293 cells; chemotaxis with CCR4-transfected L1.2 cells; calcium mobilization in CEMS529 cells and cytotoxicity in L1.2 cells) were performed using conditions as described in Ref. 5a and b. The values are reported from average of three repeats.
13. OVA lung inflammation model in mice was carried out as described in Ref. 5b.