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Optimization of 2-aminothiazole derivatives as CCR4 antagonists

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Abstract—A series of 2-aminothiazole-derived antagonists of the CCR4 receptor has been synthesized and their affinity for the receptor evaluated using a [^{125}I]TARC (CCL17) displacement assay. Optimization of these compounds for potency and pharmacokinetic properties led to the discovery of potent, orally bioavailable antagonists.

CCR4 is a G-protein-coupled receptor from the CC family of chemokine receptors that is involved in the migration of inflammatory cells into inflamed tissue.[1] CCR4 is predominantly expressed on T lymphocytes, mainly on those of the Type-2 (Th2) phenotype. CCR4 is also expressed on monocytes, macrophages, dendritic cells, and platelets.[2] The ligands for CCR4 are Thymus and Activation Regulated Chemokine (TARC, CCL17) and Macrophage-derived Chemokine (MDC, CCL22).[3] CCR4 and its ligands have been implicated in a number of inflammatory diseases including asthma,[4] psoriasis,[5] atopic dermatitis,[6] and solid organ transplant rejection.[7] Therefore, it has been postulated that CCR4 antagonists could play a beneficial role in the treatment of these diseases.

A series of 2-aminothiazole derivatives (lead structure shown in Fig. 1) was discovered as antagonists of CCR4. Compound **1** inhibited binding of radiolabeled TARC and MDC to CCR4 receptors on the surface of CEM cells[8] and also inhibited the in vitro migration of CEM cells mediated by TARC (IC_{50} = 6.4 μM).[9]

In addition to potency optimization, it was also critical to improve the pharmacokinetic properties of the lead series in order to obtain compounds suitable for evaluation in proof-of-concept experiments in vivo. The clearance of **1** in rats after a 0.5 mg/kg iv dose is high (4.2 L/h/kg) and the half-life is short (0.4 h). The bioavailability after oral gavage dosing at 2 mg/kg is 2%. In this publication, we describe the design of potent CCR4 antagonists with good pharmacokinetic properties in rats.

The thiazole-derived compounds were synthesized according to Scheme 1. Anilines were converted to thioureas by treatment with benzoylisothiocyanate followed by saponification of the benzoyl group. Subsequently, the *N*-arylthioureas were treated with α-chloroketones in refluxing methanol to obtain 2-amino-4-alkyl-substituted thiazoles (**1**–**8** and **15**–**26**). Compounds **11**–**14** were obtained by reacting the *N*-arylthioureas with 1,3-dichloroacetone in acetone at room temperature, followed by alkylation of the chloromethylthiazole intermediate with an appropriate secondary amine.

A series of compounds was synthesized that contained pyrimidine, isoxazole, and pyrazole as potential replace-

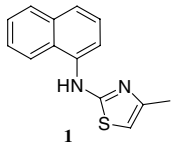

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Figure 1. Potency of lead compound **1**: CCR4/[^{125}I]TARC: IC_{50} = 1.7 μM, CCR4/[^{125}I]MDC: IC_{50} = 3 μM.

Keywords: CCR4; Chemokine; Chemokine receptor; TARC; CCL17; MDC; CCL22; GPCR.
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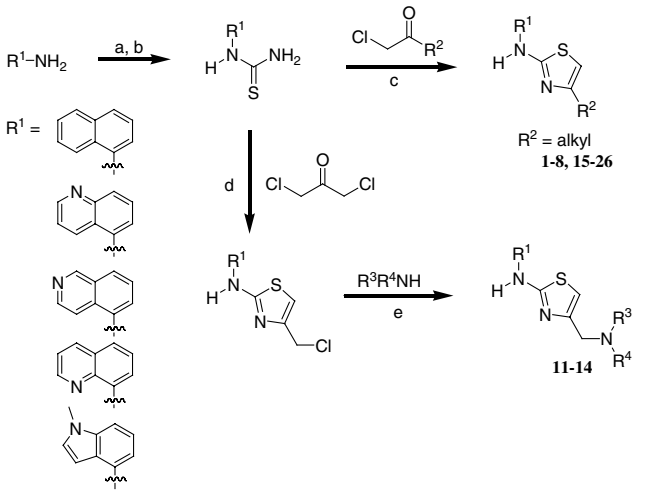
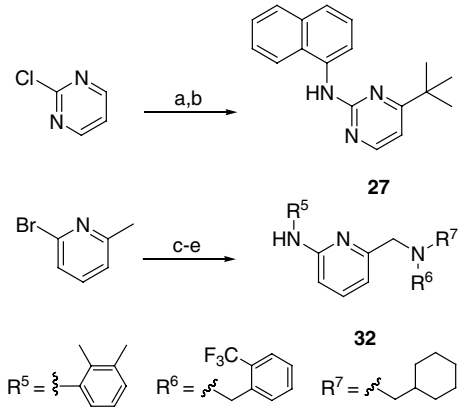


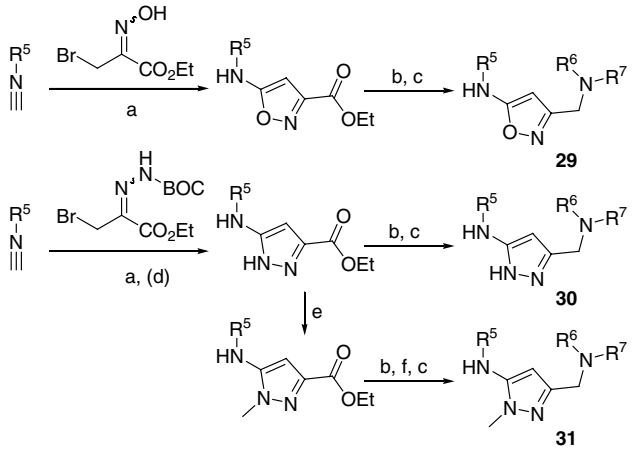
Scheme 1. Reagents and conditions: (a) benzoylisothiocyanate, acetone, rt, 1 h, 98%; (b) 10% NaOH, 98 °C, 10 min.; 90%; (c) MeOH, reflux, 3 h, 80–100%; (d) acetone, rt, overnight, 70–80%; (e) $NaHCO_3$, NaI, acetone, reflux, 2 h, 60–75%.

ments to the thiazole moiety (Schemes 2 and 3). Pyrimidine **27** was obtained by addition of *t*-BuLi to 2-chloropyrimidine followed by DDQ oxidation and then reaction with α-naphthylamine. The pyridine compound **32** was prepared by free-radical bromination of 2-bromo-6-picoline, and then displacement of the aliphatic and ring bromine atoms with appropriate amines under thermal and Pd-catalyzed conditions, respectively.

The isoxazole and pyrazole analogs (Scheme 3) were prepared from a common aryl isocyanide intermediate. Cyclization with the oxime or the BOC-protected hydrazone of ethyl bromopyruvate generated the isoxazole and pyrazole carboxy esters, respectively. In cases where the BOC group was not cleaved under the conditions of the cyclization, a quick TFA treatment provided the unprotected pyrazole. After conversion of the esters to the corresponding aldehydes, reductive amination provided compounds **29**–**31**.



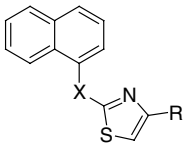
Scheme 2. Reagents and conditions: (a) *t*-BuLi, then DDQ, Et_2O, −30 °C to 0 °C, 1 h, 100%; (b) α-naphthylamine, ethylene glycol, reflux, 54%; (c) NBS, AIBN, C_6H_6, 90 °C, 24 h, 71%; (d) R^6R^7NH, Cs_2CO_3, DMF, rt, 24 h, 9%; (e) R^5NH_2, $Pd_2(dba)_3$, dppp, *t*-BuOK, $PhCH_3$, 70 °C, 24 h, 73%.

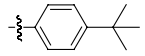
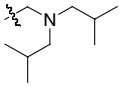
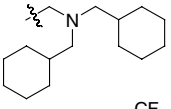
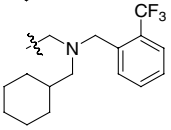


Scheme 3. Reagents and conditions: (a) Na_2CO_3, CH_2Cl_2, rt, 24 h, 35%; (b) DIBAL, $PhCH_3$, −78 °C, 30 min, 42%; (c) R^5R^6NH, $NaHB(OAc)_3$, 1,2-DCE, rt, 24 h, 22%; (d) TFA, 1 h, 100%; (e) CH_3I, Cs_2CO_3, acetone, 0 °C to rt, 24 h, 41%; (f) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, rt, 24 h, 98%.

The compound optimization effort was primarily guided by the [^{125}I]TARC ligand displacement assay.[8] The effect of replacing the linker region between the naphthalene and thiazole rings was explored first (Table 1). It was

Table 1. Thiazole derivatives as CCR4 antagonists



Compound	X	R	[^{125}I]TARC IC_{50}[a] (μM)
1	–NH–	CH_3	1.7
2	–N(CH_3)–	CH_3	>25
3	–NH–	–$CH(CH_3)_2$	0.17
4	–CH_2–	–$CH(CH_3)_2$	>25
5	–NH–	–$C(CH_3)_3$	0.08
6	–NH–	–$CH_2C(CH_3)_3$	0.22
7	–NH–	–Ph	5.5
8	–NH–		0.9
9	–NH–	–CH_2NH_2	>25
10	–NH–	–$CH_2NHCH_2CH_3$	1.0
11	–NH–	–$CH_2N(CH_2CH_3)_2$	0.8
12	–NH–		0.045
13	–NH–		0.018
14	–NH–		0.014

[a] Values are means of three experiments, standard deviation is ±30%. Displacement of [^{125}I]TARC from the CCR4 receptor expressed on CEM.[8]

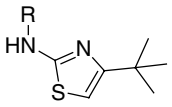
discovered that a secondary amino group is preferred over a tertiary amine or a methylene linker (compare **1** to **2** and **4**).

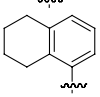
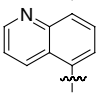
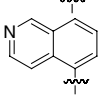
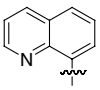
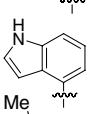
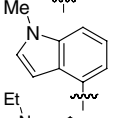
Exploration of the 4-position of the thiazole ring demonstrated that bulky alkyl groups are optimal for activity (**5**–**6**). Furthermore, alkyl substitutions are preferred over aryl substitutions (**7**–**8**). In addition, while hydrophobic substituents appear to be preferred at this position, the hydrophobic alkyl groups can be successfully replaced by bulky secondary and tertiary amines (**11**–**14**).

Concurrently with the optimization of the thiazole substituents, a series of naphthalene replacements were evaluated (Table 2). Replacement of the naphthalene moiety by unsubstituted phenyl (**15**) resulted in a significant loss of activity. While a series of phenyl analogs with substitutions at positions 2 and/or 3 (**16**–**20**) showed improved activity compared to the unsubstituted analog, none of these compounds were as potent as the naphthyl group. A series of bicyclic-heteroaromatic replacements, which included quinoline (**21**, **23**), isoquinoline (**22**), and indole (**24**–**26**) derivatives, was also explored for potential replacements of the naphthyl moiety. Among these replacements, indoles demonstrated improved potency. Thus, *N*-methyl indole **25** is more potent than the naphthyl-derived analog (**5**).

A systematic survey of thiazole ring replacements was also performed. Replacement of the thiazole ring by a pyrimidine (**27**) or unsubstituted pyrazole (**30**) resulted in the substantial loss of activity, while *N*-methyl pyrazole (**31**) and pyridine (**32**) both showed significant activity (0.37 and 0.34 μM, respectively, against hCCR4/

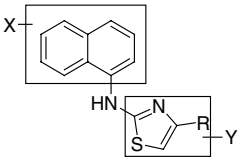
Table 2. CCR4 antagonists with varying N-substituents

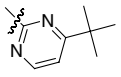
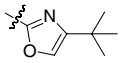
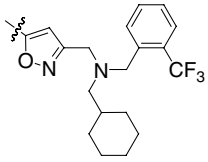
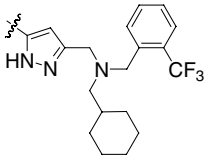
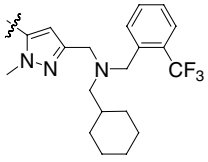
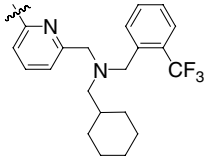


Compound	R	[^{125}I]TARC IC_{50}[a] (μM)
15		4.5
16		0.9
17		2.0
18		0.44
19		0.26
20		0.21
21		0.3
22		1.5
23		>25
24		0.49
25		0.037
26		0.21

[a] Values are means of three experiments, standard deviation is ±30%. Displacement of [^{125}I]TARC from the CCR4 receptor expressed on CEM.[8]

Table 3. CCR4 antagonists with varying modifications at the thiazole moiety



Compound	X	Y	[^{125}I]TARC IC_{50}[a] (μM)
27			>25
28			0.08
29			12.1
30			>25
31			0.37
32			0.34

[a] Values are means of three experiments, standard deviation is ±30%. Displacement of [^{125}I]-labeled TARC from the CCR4 receptor expressed on CEM.[8]

[^{125}I]TARC). However, the most successful example in this area is the oxazole replacement (**28**). Compared with the corresponding thiazole compound (**5**), the oxazole possesses comparable potency against CCR4 Table 3.

Placement of bulky hydrophobic tertiary amines at the 4-position of the thiazole led to the discovery of potent compounds with improved potency and pharmacokinetic properties. Replacement of the methyl group on **1** by an *N*,*N*-bis(cyclohexylmethyl)methyl amine moiety (**13**) led to a significant improvement in potency. Compound **13** is approximately 100-fold more potent as an inhibitor of TARC and MDC binding to CCR4 than **1** (**13**; CCR4/[^{125}I]TARC: IC_{50} = 0.018 μM; [^{125}I]MDC IC_{50} = 0.031 μM). This compound is also a functional antagonist of CCR4, as demonstrated by its ability to inhibit TARC-mediated cell migration (IC_{50} = 0.53 μM). Furthermore, compound **13** has improved pharmacokinetic properties in rats relative to compound **1**. The clearance of **13** in rats is approximately 4-fold lower than that of compound **1** (i.e., 1.07 L/h/kg vs 4.2 L/h/kg) after a 0.5 mg/kg iv dose. The terminal half-life is also improved from 0.4 to 3.6 h. The oral bioavailability of compound **13** is 63% following a 2 mg/kg po dose. Thus, compound **13** can be used as a reasonable tool to validate the CCR4 target in vivo.

In summary, a novel series of 2-aminothiazole-derived CCR4 antagonists was discovered and optimized for potency and pharmacokinetic properties. It was found that bulky hydrophobic substituents are preferred at the 4-position of the thiazole ring. These observations led to the discovery of compound **13**, which displays adequate potency and pharmacokinetic properties to be used as a tool for further study of the CCR4 receptor.

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8. Assays for CCR4 were performed using the human T lymphoblastic cell line CEM, obtained from the ATCC (CCRF-CEM variant), which expresses high levels of endogenous human CCR4. Compounds were diluted into DMSO and added to individual wells of a 96-well polypropylene assay plate. CEM cells were resuspended to 5×10^6/mL in buffer (RPMI-1640 supplemented with 0.5% BSA); 90 μL was added to each well of the assay plate. [^{125}I]TARC (Perkin-Elmer) was diluted to 100 pM in buffer; 100 μL was added to each well of the assay plate. The assay plates were incubated at room temperature for 2 h. The assay mixtures were filtered through GF/B filter plates using a FilterMate Harvester (Perkin-Elmer). Retained radioactivity was quantified by scintillation counting.
9. Migration assays for CCR4 were performed using 96-well ChemoTX migration plates (NeuroProbe) with a 5 μm pore size. Compounds were diluted into DMSO and added to individual wells of two matched 96-well polypropylene assay plates. TARC (R&D Systems) was diluted to 1 ng/mL in buffer; 200 μL was added to each well of the second assay plate to combine the TARC with compound and then 32 μL of this mixture was plated into the bottom chamber of the migration plate. The filter membrane for the migration plate was secured in place. CEM cells were resuspended to 5×10^6/mL in buffer; 200 μL was added to each well of one assay plate to combine the cells with compound, then 50 μL of this mixture was plated onto the filter membrane of the migration plate. The migration plate was transferred to a 37 °C incubator (5% CO_2, 95% humidity) and incubated for 2 h. At the end of this incubation period, the cells were aspirated from the top of the filter and the filter was removed. Alamar blue (5 μL) was added to the bottom chamber, the plate was returned to the incubator and incubated for another hour. At the end of this incubation period, the plates were read on a fluorescent plate reader (530 absorbance, 590 emission).