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α-Aminothiazole-γ-aminobutanoic amides as potent, small molecule CCR2 receptor antagonists

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Abstract—A series of racemic and homochiral α-aminothiazole-γ-aminobutyroamides that display high affinities for human and murine CCR2 and functional antagonism by inhibition of monocyte recruitment are described. A representative example is (2*S*)-2-[2-(acetylamino)-1,3-thiazol-4-yl]-*N*-[3-methyl-5-(trifluoromethyl)benzyl]-4-(4-phenylpiperidin-1-yl)butanamide, which shows 5 nM affinity for human monocytes and CHO cells expressing the human CCR2b receptor. It also inhibited MCP-1 initiated chemotaxis of human monocytes with an IC_{50} of 0.69 nM.

Chemokines are a family of small (70–200 amino acids) proinflammatory cytokines with potent chemotactic activities. Chemokine receptors have been implicated as important mediators of inflammatory and immunoregulatory disorders and diseases, including asthma, rhinitis, and allergic diseases, as well as autoimmune pathologies such as rheumatoid arthritis and atherosclerosis.[1] A subset of chemokines are potent chemoattractants for monocytes and macrophages. The best characterized of these is MCP-1 (monocyte chemoattractant protein-1 (CCL2)), whose primary receptor is CCR2.[2,3] MCP-1 specifically attracts monocytes and memory T cells. [4] Its expression occurs in a variety of diseases characterized by mononuclear cell infiltration, and there is substantial biological and genetic evidence for its essential role in inflammation.[5] CCR2 antagonism is recognized as an approach for the treatment of autoimmune diseases such as rheumatoid arthritis and multiple sclerosis.[6] Thus, the discovery and development of small molecule CCR2 antagonists has been regarded as an important pharmaceutical goal.[7]

In earlier reports,[8] we described the discovery of our first screening CCR2 antagonist lead **1** with micromolar binding affinity (IC_{50} = 0.7 μM) and identified several more potent analogs by replacement of 4-fluorophenyl group with 3-thienyl or 3-furanyl groups. However, there were concerns that the molecules lacked sufficient selectivity over the NK-1 receptor (compound **1**: NK-1 IC_{50} = 700 nM).

After systematic modification of the backbone, we improved the binding affinities to the nanomolar range (compound **2a**: IC_{50} = 34 nM). Replacement of 4-fluorophenyl ring with small aliphatic rings such as cyclopropyl resulted in more potent analogs (compound **2b**: IC_{50} = 4 nM). Unfortunately the compounds in this series lacked binding affinity toward murine CCR-2, which prevented evaluation in in vivo animal models. Accordingly, a strategy was put in place to replace the aryl/aliphatic groups with heterocycles in the hope of finding additional interactions with the receptor to further enhance potency. In this paper, we report the details of our effort on the introduction of the aminothiazole and its derivatives at the α-position of the γ-aminobutyroamide scaffold leading to a series of novel CCR2 antagonists with sub-nanomolar binding and functional affinities. In addition, some of these compounds showed potent antagonism of the mouse CCR2 receptor for the first time in this series Figure 1.

Keywords: CCR2; Antagonist; Aminothiazole; Chemotaxis; Monocyte.
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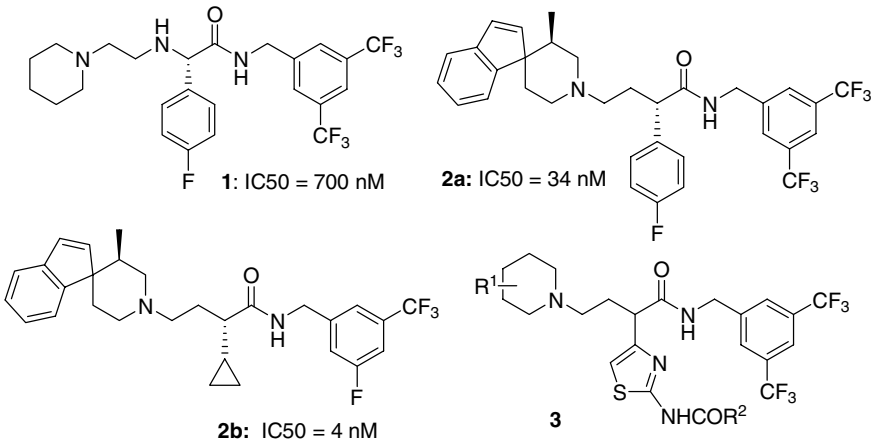


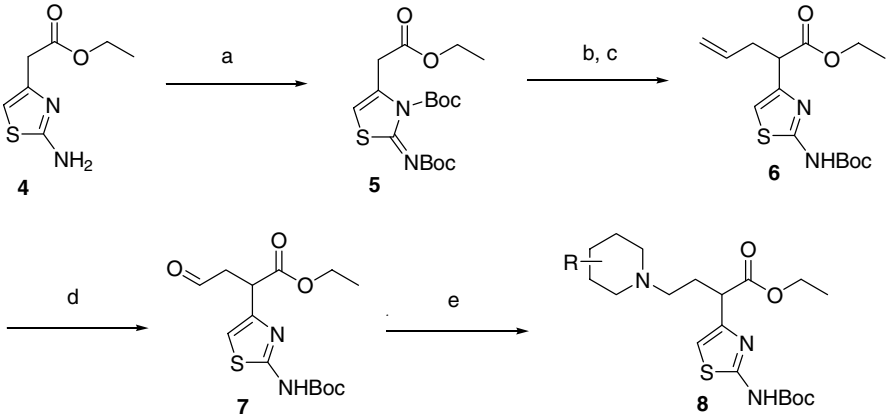
Figure 1. CCR2 antagonists.

The key intermediate γ-amino ester **8** was prepared according to the procedure described by Kang et al.[9] (Scheme 1). Commercially available ethyl (2-amino-1,3-thiazol-4-yl)acetate **4** was protected as its bis-Boc ester **5**. Subsequent allylation was accomplished by treatment of **5** with ^{n}BuLi at −78 °C, followed by allyl bromide. The resulting crude alkylated intermediate was selectively hydrolyzed to mono-Boc protected ester **6** with 30% aq citric acid. The γ-aldehyde ester **7** was prepared by oxidation of the alkene ester **6**. Reductive aminations of the aldehyde **7** with amines gave the γ-amino ester **8**.

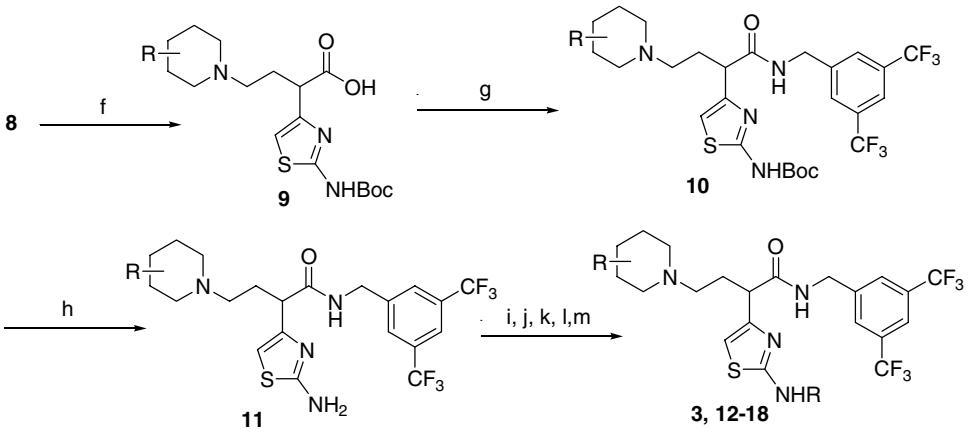
Hydrolysis of the γ-amino ester **8** (Scheme 2) was carried out in aqueous MeOH with lithium hydroxide. The crude reaction mixture was passed through a silica gel column, eluting with methanol/methylene chloride to give the amino acid **9**. The coupling of the amino acid with bis-trifluoromethyl benzylamine hydrochloride salt was mediated by EDAC without added base, affording the Boc-protected α-aminothiazole-γ-aminobutanoic amide **10**. Removal of the Boc group in **10** with neat TFA yielded the free aminothaizole **11**, which underwent various standard derivatizations to give the final compounds **3**, and **12**–**18**.

It was found that racemic **10** could be easily separated into two single enantiomers on chiral HPLC in most cases. TFA-mediated deprotection then gave the corresponding enantiomerically pure intermediates **11**, which could be converted into the final compounds **19**–**25** as single enantiomers. In some cases, direct separation of racemic final products was possible; for examples, almost all the compounds from the spiroindenyl-piperidine series were separable on chiral HPLC (OD Column, eluting with 10% EtOH/hexane).

All the compounds prepared above were evaluated for their ability to inhibit MCP-1 binding to membranes of human monocytes stably expressing CCR2 in the presence of 0.5% BSA. For functional studies, inhibition of MCP-1-stimulated chemotaxis in freshly isolated peripheral human monocytes was evaluated (CTX assay). The binding displacement data and the chemotaxis data are expressed as IC_{50} values.

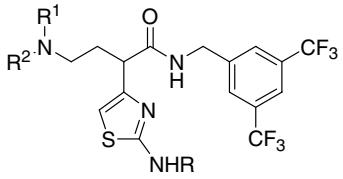


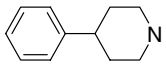
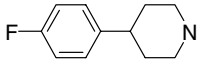
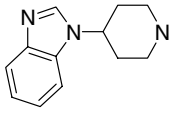
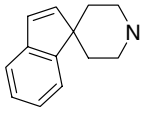
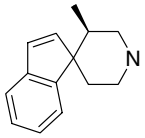
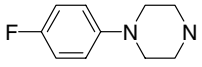
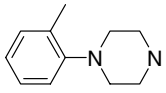
Scheme 1. Reagents and conditions: (a) Boc_2O (2 equiv)/DMSO, 5 days, the product precipitated out of the solution, 82%; (b) ^{n}BuLi (1.1 equiv)/THF/−78 °C, allyl bromide (2.0 equiv)/−78 °C to RT; (c) 30% aq citric acid, RT, overnight, 67% (two steps); (d) NMO (1.1 equiv)/OsO_4 (cat.)/acetone/water (1:1), then $NaIO_4$ (1.2 equiv)/MeOH/water, 52% (two steps); (e) amine (1.2 equiv)/DIEA (1.0 equiv)/$NaBH(OAc)_3$ (1.2 equiv), Molecular Sieves (4A)/DCM, overnight.



Scheme 2. Reagents and conditions: (f) LiOH·H_2O (1.5 equiv)/MeOH, reflux, 2 h; (g) 3,5-bis-trifluoromethylbenzyl amine hydrochloride (1.0 equiv)/EDAC·HCl (2.0 equiv)/DCM; (h) 50% TFA/DCM; (i) for the synthesis of amide: $(RCO)_2O$ (1.2 equiv)/Py/DCM; (j) for the synthesis of urea: RN=C=O (1.2 equiv)/DCM; (k) for the synthesis of guanidine: pyrrolyl–C(=NH)NH_2 (2 equiv), 220 °C, 30 min; (l) for the synthesis of carbamide: ClCOOR (1.2 equiv)/Py/DCM; (m) for the synthesis of sulfonamide: $(RSO_2)O$ (1.5 equiv)/Py/DCM.

Table 1. Binding affinity of compounds at hCCR2[10,11]



NR^1R^2	Compound	IC_{50} (nM) R = H	Compound	IC_{50} (nM) R = COMe
	**11a**	20	**3a**	5.2
	**11b**	9.1	**3b**	1.8
	**11c**	28.6	**3c**	3.0
	**11d**	7.3	**3d**	1.7(0.7)[a]
	**11e**	1.3	**3e**	3.0(0.6)[a]
	**11f**	70%[b]	**3f**	32.7
	**11g**	182.7	**3g**	14.5
	**11h**	75% [b]	**3h**	13

[a] IC_{50}: Chemotaxis, human MCP-1 Monocyte.
[b] Inhibition at 1 μM.

Our initial effort starting from 4-phenylpiperidine led to the synthesis of the precursor **10a** which displayed similar binding affinity toward hCCR2 as that of the lead compound **2**. After removal of the protecting Boc group, more active **11a** (IC_{50} = 20 nM) was obtained. When compound **11a** was converted into its acetamide **3a**, the binding affinity improved to 5.2 nM (a 4-fold increase). Encouraged by these results, various aminothiazoles **11b**–**11h** and acetamidothiazoles **3b**–**3h** were prepared by incorporating other piperidines and piperazines into the gamma position. The results are summarized in Table 1.

The head-to-head comparison between the free aminothiazoles **11** and acetamidothiazoles **3** showed that the binding affinities of **11** were much weaker than those of **3**. The most potent compounds were obtained when phenylpiperidines and spiroindenepiperidines were used as the amine moieties. Although the aminothiazoles from piperazines such as **11f**–**11g** generally showed only micromolar binding affinities, their respective acetamides **3f**–**3g** were quite potent. The potency of compounds **11d** and **11e** was further confirmed by the CTX assay (IC_{50} = 0.7 and 0.6 nM, respectively). The effect of acetylation was also quite obvious in the simple piperidine cases (**11h** and **3h**). The above results may imply a binding pocket of the CCR2 receptor being reached by acetyl group.

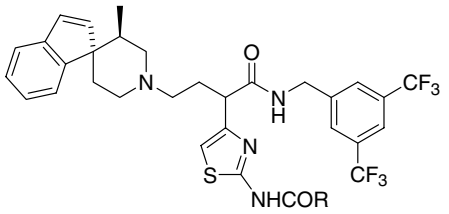
A more detailed study involving derivatization of the free aminothiazole **11a** showed that most of the amides, ureas, and even guanidines displayed similar potent antagonism toward the hCCR2 receptor. In the amide series, smaller groups gave higher potency. For example, larger amides were less potent than smaller ones ($CO^tBu < COPh < CO^iPr < CO^cPr < COEt$). In addition, methanesulfonamide **3s** was much less active compared with the amide **3a**. These results are summarized in Table 2.

One exception to the structure-activity trend was that no binding affinity improvement was observed when compound **11e** was converted into amide **3e**. None-the-less, murine CCR2 antagonism was observed in this series when amides were incorporated, providing pharmacological tools for in vivo animal model studies. The results are summarized in Table 3.

The spiroindenylpiperidine part of the compounds **12**–**18** seems important for the murine CCR2 activities too. Much weaker mCCR2 antagonism (IC_{50} > 1 μM) was observed for the compounds without spiroindenylpiperidine substructure.

As expected, the binding affinity of one of the two enantiopure isomers was much higher than the other one. Table 4 lists a few examples of single enantiomers purified by preparative chiral HPLC. About a 2-fold increase in potency was observed in most cases when compared to the racemic analogs.

Table 3. Binding Affinity of compounds at hCCR2 and mCCR2[10]



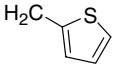
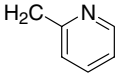
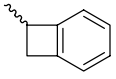
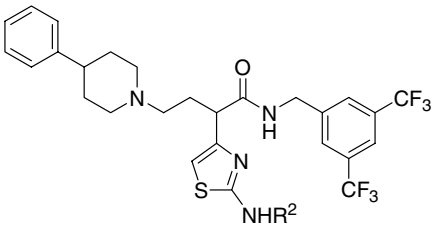
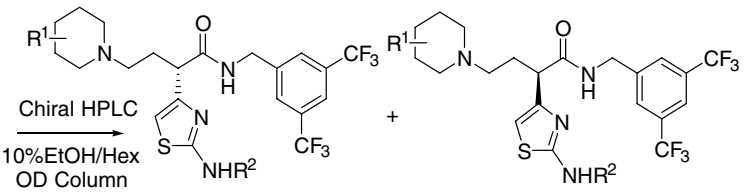
Compound	R	IC_{50} (nM)	
		hCCR-2	mCCR-2
12	Ph	17	952
13	CH_2Ph	19	165
14		23	81
15		**13**	**79**
16	3-Pyridyl	14	186
17	CHMePh	74	517
18		36	111

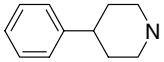
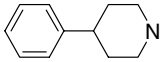
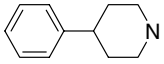
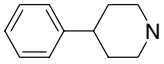
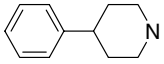
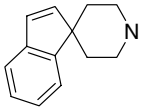
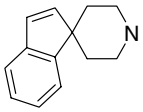
Table 2. Binding affinity of compounds at hCCR2[10]



Compound	R^2	IC_{50} (nM)	Compound	R^2	IC_{50} (nM)
3a	COMe	5.2	**3n**	COPh	16
3i	CHO	4.0	**3o**	CONHMe	3.0
3j	COEt	3.5	**3p**	$CONMe_2$	2.3
3k	CO^cPr	3.9	**3q**	COOMe	2.6
3l	CO^iPr	7.4	**3r**	$C(=NH)NH_2$	1.3
3m	CO^tBu	18	**3s**	SO_2Me	36

Table 4. Binding affinity of enantiopure compounds at hCCR2[10,11]



Compound	R^1	R^2	IC_{50} (nM)	
			A	**B**
19		Boc	14.3	13.3
20		H	13.3	229
21		COMe	1.8 (**0.7**)[a]	42
22		COOMe	3.5	85% at 1 μM
23		CONHMe	**0.43** (1.8)[a]	30(87)[a]
24		COMe	1.4 (5.3)[a]	6.5(27)[a]
25		CONHMe	**0.8**	5.8

[a] IC_{50}: Chemotaxis, human MCP-1 Monocyte.

The selectivity of these new CCR2 antagonists over other chemokine receptors was generally quite good. In addition, the NK-1 selectivity was greatly improved compared with our original lead **1**. The selectivity of compound **21** is shown in Table 5.

Table 5. Binding affinities of compound **21** over other chemokine receptors

CCR	CCR-1	CCR-3	CCR-4	CXCR4	CCR-5	NK-1
IC_{50}[a]	66%	7%	86%	27%	44%	56%

[a] % inhibition at 1 μM (IC_{50} not determined).

Compound **21** can be orally absorbed but it was cleared rapidly. In general, these compounds suffered from poor pharmacokinetic profiles. The PK data in rats and dogs for compound **21** are shown in Table 6.

In summary, we have prepared a series of novel α-aminothiazole-γ-aminobutyroamides that show good hCCR2 and/or mCCR2 binding potency, excellent chemotaxis inhibitory activity, and selectivity over other chemokine receptors. Studies on further optimization of in vitro and in vivo properties will be reported in due course.

Table 6. Pharmacokinetic profiles of compound **21**

PK	F[a] (%)	$T_{1/2}$ (h)	CL ([b])	V_d (l/kg)	AUCN po/iv (μM h)	C_{max} (μM)	T_{max} (h)
Rat	17	1.4	52.5	5.3	0.13/0.71	0.064	2.2
Dog	1.6	2.28	21.6	4.0	0.03/1.79	0.018	0.5

[a] Doses: male rat Sprague–Dawley (iv: 1.0 mpk; oral: 3 mpk); Male Beagle Dog (iv: 0.20 mpk; oral: 3.0 mpk).
[b] ml/min/kg.

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10. Receptor affinity in a human CCR2 binding assay was determined by measuring inhibition of ^{125}I-MCP-1 binding to monocyte cells stably transfected with CCR2 in the presence of 0.5% BSA.
11. The chemotaxis assay was performed according to the procedure described in Ref. 8a.