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Capped diaminopropionamide–glycine dipeptides are inhibitors of CC chemokine receptor 2 (CCR2)

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Abstract—A new series of CCR2 antagonists has been discovered that incorporates intramolecular hydrogen bonding as a strategy for rigidifying the scaffold. The structure–activity relationship was established through initial systematic modification of substitution pattern and chain length, followed by independent optimization of three different substituents (benzylamine, carboxamide, and benzamide). Several of the acyclic compounds display 10–30 nM binding affinity for CCR2. Moreover, these antagonists are able to block both MCP-1-induced Ca^{2+} flux and monocyte chemotaxis, and are selective for binding to CCR2 over CCR1 and CCR3.

CC chemokine receptor 2 (CCR2) is the primary chemokine receptor on monocytes recruited to sites of inflammation;[1] it is also the exclusive receptor for monocyte chemoattractant protein-1 (MCP-1), a well-characterized pro-inflammatory cytokine. Targeting this pro-inflammatory pair through both genetic and pharmacologic approaches reduces inflammation in classic rodent models of rheumatoid arthritis, multiple sclerosis, and atherosclerosis.[2] These data, together with the fact that both $CCR2^{-/-}$ and $MCP\text{-}1^{-/-}$ are viable, fertile, and devoid of any overt phenotype, have prompted researchers to pursue CCR2 antagonism as an approach toward treating inflammatory diseases.[2,3]

Others have disclosed that cyclic diamines such as 3-aminopyrrolidine and 3-aminomethylpiperidine could serve as scaffolding elements for potent CCR2 antagonists (binding IC_{50}'s < 100 nM).[4] We postulated that the 3-aminopyrrolidine subunit could be replaced with an *acyclic* diamine motif, provided that a functional group capable of intramolecular hydrogen bonding was suitably positioned along the diamine backbone (Fig. 1). In this Letter, we describe our initial test of this hypothesis.

Scheme 1 illustrates representative examples of the straightforward chemistry used to synthesize these molecules.[5] The commercially available N_α-Boc, N_ω-Cbz diaminoacids were esterified or amidated. The resulting products were deprotected (TFA) and coupled with a substituted hippuric acid. Hydrogenolysis of the Cbz and reductive amination provided final products. In the case of diaminopropionic acid (Dap)-derived products ($n = 1$), the compounds could be derivatized further through reduction, hydrolysis, or amidation. Longer chain lengths ($n = 2$ and 3) required that the amide be introduced in the first step in order to avoid intramolecular cyclization in the late-stage coupling. Since the

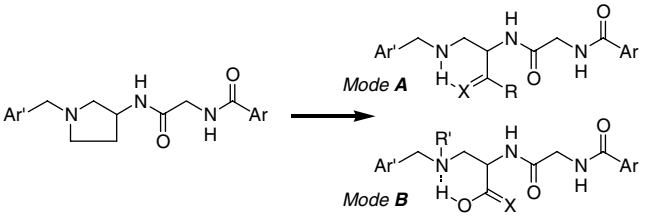

Figure 1. Use of intramolecular hydrogen bonding to constrain the conformation of an acyclic scaffold while introducing additional specificity/recognition elements.

Keywords: CCR2; Small molecule; Antagonist; G-protein coupled receptor; Chemokine; Inflammation.
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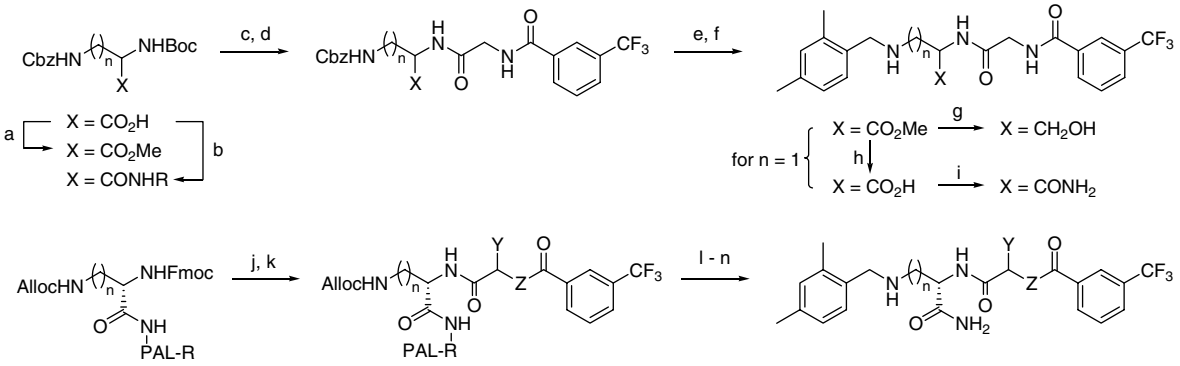


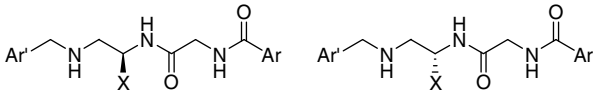
Scheme 1. Representative solution- and solid-phase synthesis of acyclic CCR2 inhibitors. Non-standard abbreviations: PAL-R = PAL polystyrene resin. Reagents and conditions: (a) EDC, MeOH; (b) H_2NR, HATU, *i*-Pr_2NEt, CH_2Cl_2/DMF; (c) TFA, CH_2Cl_2; (d) $HO_2CCH_2NHCO(3\text{-}CF_3Ph)$, BOP, *i*-$Pr_2NEt$, CH_2Cl_2/DMF; (e) H_2, 5% Pd/C (Degussa), MeOH; (f) 2,4-Me_2PhCHO, $NaCNBH_3$, MeOH; (g) $LiBH_4$, MeOH, THF; (h) LiOH, THF/MeOH/H_2O; (i) H_3N/MeOH, HATU; (j) piperidine/NMP wash, then Fmoc-amino acid, HBTU, HOBt, *i*-Pr_2NEt, NMP; (k) piperidine/NMP wash, then $HO_2C(3\text{-}CF_3Ph)$, HBTU, HOBt, *i*-Pr_2NEt, NMP; (l) $Pd(PPh_3)_4$, 37:2:1 $CHCl_3$/AcOH/*N*-methyl morpholine; (m) 2,4-Me_2PhCHO, $NaCNBH_3$, 1% AcOH/dimethylacetamide; (n) 95:5 TFA/H_2O, triethylsilane.

primary amide derivatives proved to be potent inhibitors (see Table 1), we employed solid-phase peptide synthesis protocols to access some compounds.[6] This approach allowed for the rapid variation of all regions of the scaffold from the starting N_α-Fmoc, N_ω-alloc diaminoacids (Scheme 1).

Table 1 lists the CCR2 binding data obtained with a pilot set of compounds derived from Dap. The 2,4-dimethylbenzyl group, glycine spacer, and 3-trifluoromethylbenzamide grouping were standardized for the initial study. Three observations stand out: (1) the inhibitors derived from (*S*)-Dap are more potent than those from (*R*)-Dap; and (2) sidechain amides are more potent than the methyl ester, carboxylic acid, and primary alcohol, with the large activity difference between the isosteric primary amide and carboxylic acid being particularly noteworthy; (3) the compounds with simple alkyl sidechains (**1g**, **1h**) are at least 10-fold less active than their functionalized counterparts.

To test the hypothesis further, we synthesized a group of compounds that altered the proposed hydrogen bonding inherent in the diaminopropionic acid subunit (Fig. 2). Alternate positioning of the amide or ester grouping along the diaminoethane moiety provides inhibitors with weaker potency (see **3a**, **3b** in Fig. 2;[7] the (*S*)-variant of an analog of **3b** exhibits similar activity, data not shown). Inhibitors derived from elongation of either the sidechain or main chain are less potent than their Dap analogs (cf. **2** and **4**, **5**; analogs from longer main chain lengths are weaker still). Notably, the rank-order activity for the three surveyed amide sidechain groups is altered as the length of the main chain increases (cf. **2d**–**2f** and **5d**–**5f**). Furthermore, amine methylation exerts differential effects on products from the Dap ($n = 1$) and diaminobutyric acid (Dab, $n = 2$) scaffolds:

Table 1. Initial test of the hypothesis shown in Figure 1 through probing of sidechain identity and absolute stereochemistry



1 Ar' = 2,4-Me_2Ph, Ar = 3-CF_3Ph **2**

Series, X group	CCR2 binding IC_{50}[a] (μM)	
	1	**2**
a, CO_2Me	1.0 ± 0.9 (2)	1.5 ± 0.5 (2)
b, CH_2OH	2.2 ± 0.1 (2)	0.32 ± 0.22 (2)
c, CO_2H	31% I at 10 μM	5.5 ± 1.8 (2)
d, $CONH_2$	0.63 ± 0.10 (2)	0.066 ± 0.005 (4)
e, CONHEt	0.56 ± 0.29 (2)	0.11 ± 0.04 (2)
f, CONH-*t*-Bu	0.11 ± 0.03 (3)	0.048 ± 0.011 (13)
g, Me	35% I at 10 μM	Not tested
h, *i*-Pr	20% I at 10 μM	Not tested

[a] Binding was performed using 0.3 nM [^{125}I]MCP-1 and human peripheral blood mononuclear cells at 23 °C (Ref. 5). IC_{50} values (*n*) are reported as mean ± SD ($n = 2$) or mean ± SEM ($n > 2$). For less potent compounds, % inhibition (% I) at a fixed inhibitor concentration is listed ($n = 1$).

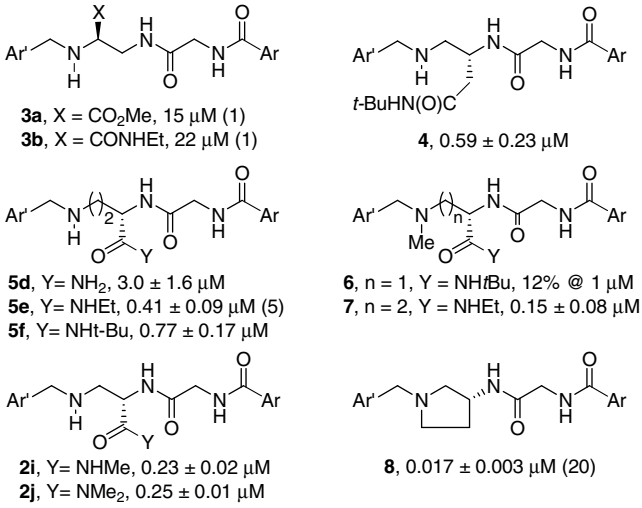
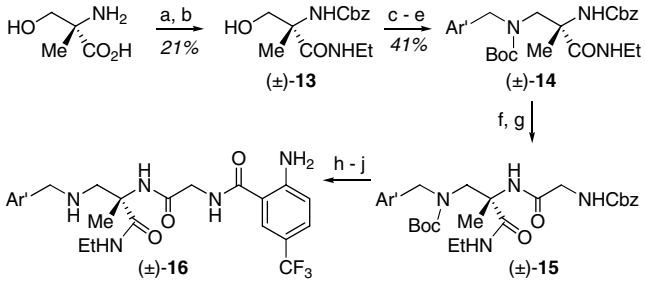


Figure 2. Additional tests of the hypothesis shown in Figure 1. CCR2 binding IC_{50} values are listed as described in the footnote to Table 1 ($n = 2$ unless indicated). Ar = 3-CF_3–phenyl and Ar′ = 2,4-$(Me)_2$–phenyl.

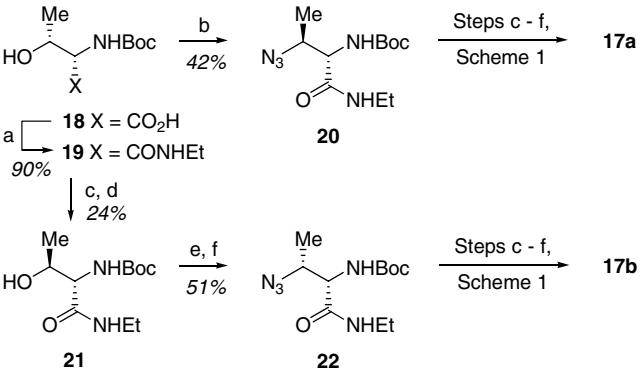
in the (*S*)-Dab series, tertiary amines are more active than their secondary counterparts, whereas tertiary amines are >10-fold less active than their secondary counterparts in the (*S*)-Dap series (cf. **2f** and **6** vs **5e** and **7**; several other examples in both series exhibit similar shifts, data not shown). Note, however, *N*-methylation of the *amide* is tolerated in the (*S*)-Dap series (cf. **2i** and **2j**).

Taken together, Table 1 and Figure 2 show that acyclic *N*-benzylated Dap–Gly dipeptides are potent CCR2 antagonists. Indeed, the binding potency of **2f** approaches that of cyclic lead structure **8**. Collectively, the data are consistent with the involvement of the intramolecular hydrogen bonding postulated in Figure 1, with Mode A being optimum (sidechain acts as H-bond acceptor). This conclusion is also supported by conformational modeling in water, in which the internal H-bond was found in >99.8% of Boltzmann-weighted population (see Supporting Information). An alternative hypothesis is that the amide sidechain moiety interacts beneficially with CCR2.

We continued our study by examining conservative modifications of both the Dap and Gly subunits. While the synthesis of the majority of the Gly analogs was facilitated by employing the aforementioned solid-phase approach using PAL resin as the support (Scheme 1), the backbone methylation scan of the (*S*)-Dap subunit required that we synthesize specialized starting materials (Schemes 2 and 3).[5] The synthesis of the α-methyl compound **16** proceeded from the commercially available (±)-α-methyl serine (Scheme 2); the choice of synthetic route was ultimately dictated by problems relating to the steric congestion of the α-alkyl amino acid. The syntheses of the β-methyl compounds **17a** and **17b** began from L-threonine (Scheme 3). Amide formation followed by Mitsunobu inversion of the hydroxyl with hydrazoic acid[8] provided the α, β-diaminobutyramide equivalent **20**; the complementary stereoisomer **22** was produced via a double inversion sequence (**19** → **21** → **22**).[9] Compounds **20** and **22** were carried forward using the chemistry shown in Scheme 1.



Scheme 2. Synthesis of α-methyl compound **16**. Abbreviations: Ar = (2,4-Me_2)-phenyl. Reagents and conditions: (a) Cbz_2O, NEt_3, THF/H_2O; (b) $EtNH_2$, HATU, DMAP, CH_2Cl_2; (c) Dess–Martin periodinane, Pyr, CH_2Cl_2; (d) 2,4-$Me_2PhCH_2NH_2$, $NaCNBH_3$, MeOH; (e) Boc_2O, NEt_3, THF/H_2O; (f) 1 atm H_2, 5% Pd/C (Degussa), MeOH; (g) HO_2CCH_2NHCbz, HATU, *i*-Pr_2NEt, cat. DMAP, CH_2Cl_2; (h) 1 atm H_2, 5% Pd/C (Degussa), MeOH; (i) HO_2C(2-NH_2,5-CF_3)Ph, HATU, *i*-Pr_2NEt, CH_2Cl_2; (j) TFA/CH_2Cl_2.



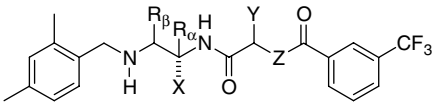
Scheme 3. Synthesis of β-methyl compounds **17a** and **b**. Reagents and conditions: (a) $EtNH_2$, BOP, CH_2Cl_2; (b) DEAD, Ph_3P, HN_3, PhH; (c) DEAD, Ph_3P, HO_2C(*p*-NO_2Ph), PhH; (d) LiOH, 2:2:1 THF/MeOH/H_2O; (e) Ms_2O, *i*-Pr_2NEt, CH_2Cl_2; (f) NaN_3, DMSO, 65 °C.

As illustrated in Table 2, conservative modification of the Dap and Gly does not enhance the potency of the inhibitors. Methyl substitution on the α-carbon (**10**, **11**) of the glycine leads to reductions in CCR2 binding, as does substitution of the Gly α-nitrogen for carbon (**12**). The methyl scan of the Dap subunit reveals that only substitution of a β-methyl group in the (*R*)-configuration (**17b**) does not impact the binding affinity substantially. The data in Table 2 may suggest that these inhibitors bind to CCR2 in a conformation that requires the wide Φ, Ψ-tolerance of the unsubstituted amino acids.

Since the unmodified (*S*)-Dap–Gly backbone appeared optimal for CCR2 binding, we focused our efforts on improving the affinity of series **2** through modification of the three capping groups: benzylamine, carboxamide, and benzamide. These groups were readily altered via chemistry analogous to that shown in Scheme 1,[5,10] beginning with N_α-Boc, N_β-Cbz (*S*)-Dap. Table 3 summarizes the remainder of the SAR profile of the carboxamide moiety. Although *tert*-butyl amide **2f** is more potent than primary amide **2d**, other simple alkyl amides and *tert*-alkyl amides are not as potent (cf. **2f** and **2k**–**2o**). Tertiary amides (see **2j**, Fig. 2, and **2y**–**2aa**, Table 3) likewise offer no advantage. Cycloalkyl amides **2p**–**2s** exhibit similar potencies to each other (90–100 nM), despite large differences in lipophilicity, but phenyl amide **2t** is 10-fold weaker (1200 nM). The apparent intolerance toward aromatic rings is also evident in the 100-fold potency difference between benzyl amide **2u** and cyclopropylmethyl amide **2w**. The 2,2,2-trifluoroethyl amide **2x** shows similar potency to **2w** and *tert*-butyl **2f**. Notably, 2,2,2-trifluoroethylamide also serves as an effective *tert*-butylamide replacement in a series of HIV protease inhibitors.[11]

Initial examination of the benzylamine moiety provided guidance for further exploration (data not shown): (1) removal of the benzyl grouping eliminates CCR2 activity; (2) carbamates, sulfonamides, and amides are inactive, independent of *ortho*-substitution on the phenyl ring; and (3) methyl substitution in either stereochemical configuration on the benzyl methylene is tolerated, but

Table 2. Modification of the (*S*)-Dap–Gly dipeptide backbone

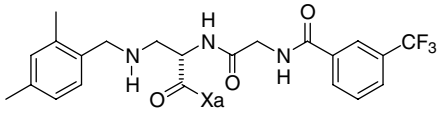


Compound	Rβ	Rα	X[a]	Y	Z	CCR2 binding IC_{50}[b] (μM)
2d	H	H	$CONH_2$	H	NH	0.066 ± 0.005 (4)
10	H	H	$CONH_2$	(*R*)-Me	NH	40% I at 10 μM
11	H	H	$CONH_2$	(*S*)-Me	NH	9% I at 10 μM
12	H	H	CONHEt	H	CH_2[c]	7% I at 10 μM
2e	H	H	CONHEt	H	NH	0.11 ± 0.04 (2)
16	H	Me[a,c]	CONHEt[a]	H	NH	8.7 (1)
17a	(*S*)-Me	H	CONHEt	H	NH	2% I at 10 μM
17b	(*R*)-Me	H	CONHEt	H	NH	0.13 ± 0.06 (2)

[a] All amide groups are in the (*S*)-configuration, save compound **16**, which is racemic.
[b] Binding was performed using 0.3 nM [^{125}I]MCP-1 and human peripheral blood mononuclear cells at 23 °C (Ref. 5). IC_{50} values (*n*) are reported as mean ± SD (*n* = 2) or mean ± SEM (*n* > 2). For less potent compounds, % inhibition (% I) at a fixed inhibitor concentration is listed (*n* = 1).
[c] The 3-trifluoromethylbenzamide was replaced with 2-amino-5-trifluoromethylbenzamide, which confers ~3-fold higher activity (see Table 5).

not enhancing, regardless of whether or not an *ortho*-substituent is present on the phenyl ring. Accordingly, we synthesized approximately fifty simple benzylamines; the highlights of this investigation are presented in Table 4. Inhibitors containing either the 2-methylbenzyl or 4-methylbenzyl group display binding affinities that are within 2-fold of each other, but ~20-fold lower than antagonists containing the 2,4-dimethylbenzyl group (cf. **2f** and **23**, **24**), suggesting an additive effect. Notably, the 10- to 20-fold enhancing effect of the *ortho*-methyl group is general for a variety of *para*-substituents (cf. **29**–**32** and **34**–**37**). The disubstituted analogs follow the same rank order as the 4-substituted analogs. Other comparisons reveal additional steric and electronic requirements for the benzylamine moiety: (1) while ethyl is allowed at the *ortho* position, methoxy is not preferred there (cf. **24**, **25** vs **26**, **35**); (2) electron-withdrawing groups are not preferred at the *para*-position, (see **27**, **28**, **33**; a larger scan confirms this, data not shown); (3) *para*-substituents larger than ethyl are poorly tolerated (see **43**, **44**; a larger scan confirms this, data not

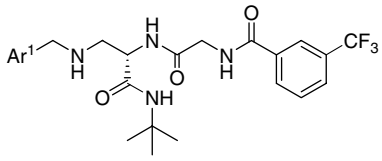
Table 3. Modification of the carboxamide moiety



Compound	Xa	CCR2 binding IC_{50}[a] (μM)
2k	NH-*i*-Pr	0.17 ± 0.03 (2)
2l	NH-*s*-Bu	0.12 ± 0.05 (2)
2m	$NHC(Me)_2Et$	0.12 ± 0.04 (2)
2n	$NHC(Me)_2CH_2OH$	0.19 ± 0.07 (5)
2o	NH(α-Me)-*c*-Pr	0.10 ± 0.08 (2)
2p	NH-*c*-Pr	0.088 ± 0.034 (2)
2q	NH-*c*-Bu	0.097 ± 0.054 (2)
2r	NH-*c*-Pent	0.099 ± 0.029 (5)
2s	NH-*c*-Hex	0.15 ± 0.01 (2)
2t	NHPh	1.2 ± 0.8 (2)
2u	$NHCH_2Ph$	7.0 ± 0.3 (2)
2v	$NHCH_2CH{=}CH_2$	0.20 ± 0.01 (2)
2w	$NHCH_2$-*c*-Pr	0.060 ± 0.018 (2)
2x	$NHCH_2CF_3$	0.055 ± 0.004 (4)
2y	Pyrrolidine	0.26 ± 0.10 (2)
2z	Morpholine	0.49 ± 0.12 (2)
2aa	*N*(OMe)Me	0.11 ± 0.03 (4)

[a] Binding was performed using 0.3 nM [^{125}I]MCP-1 and human peripheral blood mononuclear cells at 23 °C (Ref. 5). IC_{50} values (*n*) are reported as mean ± SD (*n* = 2) or mean ± SEM (*n* > 2). For less potent compounds, % inhibition (% I) at a fixed inhibitor concentration is listed (*n* = 1).

Table 4. Representative SAR of the benzylamine substituent



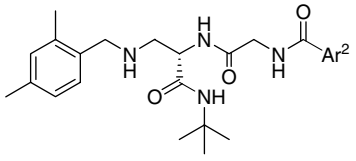
Compound	Ar^1	CCR2 binding IC_{50}[a] (μM)
2f	2,4-Me_2–Ph	0.048 ± 0.011 (13)
23	4-Me–Ph	0.75 ± 0.13 (2)
24	2-Me–Ph	0.40 ± 0.17 (2)
25	2-Et–Ph	0.38 ± 0.05 (4)
26	2,4-$(MeO)_2$–Ph	37% I at 1.0 μM
27	4-CO_2Me–Ph	20% I at 1.0 μM
28	4-SO_2Me–Ph	7.2 (1)
29	4-Br–Ph	1.7 ± 1.1 (2)
30	4-MeO–Ph	1.1 ± 0.1 (4)
31	4-Et–Ph	0.53 ± 0.41 (2)
32	4-Me_2-*N*–Ph	0.43 ± 0.18 (2)
33	2-Me,4-NC–Ph	0.90 ± 0.27 (3)
34	2-Me,4-Br–Ph	0.16 ± 0.02 (2)
35	2-Me,4-MeO–Ph	0.047 ± 0.009 (2)
36	2-Me,4-Et–Ph	0.036 ± 0.001 (2)
37	2-Me,4-Me_2N–Ph	0.036 ± 0.001 (2)
38	3-F,4-Me–Ph	27% I at 1.0 μM
39	3-O_2-*N*,4-Me–Ph	0.67 ± 0.51 (2)
40	3-H_2-*N*,4-Me–Ph	0.12 ± 0.05 (2)
41	2,3-Me_2,4-MeO–Ph	0.69 (1)
42	2,5-Me_2,4-MeO–Ph	1.3 (1)
43	4-(*n*-Bu)–Ph	5.6 (1)
44	4-Ph–Ph	30% I at 1.0 μM

[a] Binding was performed using 0.3 nM [^{125}I]MCP-1 and human peripheral blood mononuclear cells at 23 °C (Ref. 5). IC_{50} values (*n*) are reported as mean ± SD (*n* = 2) or mean ± SEM (*n* > 2). For less potent compounds, % inhibition (% I) at a fixed inhibitor concentration is listed (*n* = 1).

shown); (4) electron-withdrawing groups are also not preferred at the *meta*-position (see **38**–**40**), and even methyl substitution is modestly detrimental (cf. **35** and **41**, **42**). Although a number of potent benzylamines are identified by this scan (Table 4), none clearly surpass the initial 2,4-dimethylbenzylamine in CCR2 binding affinity.

We examined approximately forty different benzamides and highlights of this investigation are presented in Table 5; more so than the benzylamine, our survey of the benzamide was influenced by unpublished work in a separate series.[12] Replacing the 3-CF_3 substituent with less hydrophobic groups is detrimental, but the more lypophilic pentafluoroethyl group does not improve activity (cf. **2f** and **46**–**49**). Anthranilamide **50** and *meta*-aniline **51** both exhibit improved affinity relative to benzamide **2f**. The *ortho*-amine also improves the potency of the *meta*-trifluoromethoxy benzamide (cf. **47**, **52**). As expected, replacement of the 3-CF_3 benzamide with the 2-NH_2, 5-CF_3 benzamide enhances the activity of analogs containing alternative amides and benzylamines (see **65**, **66**, Table 6). Likewise, both the 2-NH_2, 5-CF_3 benzamide and 3-NH_2, 5-CF_3 benzamide enhance the activity of the (*S*)-Dab-based series (i.e., analogs of **5f**). However, none of the Dab-based inhibitors exhibit equivalent potency to the best Dap-based inhibitors (data not shown).

Table 5. Representative SAR of the benzamide moiety



Compound	Ar^2	CCR2 binding IC_{50}[a] (μM)
2f	3-CF_3Ph	0.048 ± 0.011 (13)
46	3-CF_2CF_3Ph	0.065 ± 0.015 (2)
47	3-OCF_3Ph	0.19 ± 0.06 (3)
48	3-SCF_3Ph	0.15 ± 0.03 (2)
49	3-CF_2HPh	0.40 ± 0.18 (2)
50	2-NH_2,5-CF_3Ph	0.011 ± 0.004 (7)
51	3-NH_2,5-CF_3Ph	0.023 ± 0.000 (2)
52	2-NH_2,5-OCF_3Ph	0.028 ± 0.019 (2)
53	2-NHEt,5-CF_3Ph	0.013 ± 0.003 (4)
54	2-NHBn,5-CF_3Ph	0.019 ± 0.007 (2)
55	2-NH-*i*-Bu,5-CF_3Ph	0.023 ± 0.004 (2)
56	2-($NHCO_2$-*i*-Pr),5-CF_3Ph	0.065 ± 0.004 (2)
57	2-(NHCONH-*i*-Pr),5-CF_3Ph	0.015 ± 0.001 (2)
58	2-(NHCO-*c*-Hex), 5-CF_3Ph	0.090 ± 0.016 (2)
59	3-NHMe,5-CF_3Ph	0.016 ± 0.008 (2)
60	3-NHEt,5-CF_3Ph	0.019 ± 0.008 (2)
61	3-NHBn,5-CF_3Ph	0.11 ± 0.07 (2)
62	3-NH-*i*-Bu,5-CF_3Ph	0.13 ± 0.06 (3)
63	3-($NHCO_2$Et),5-CF_3Ph	0.31 ± 0.10 (2)
64	3-(NHCO*c*-Hex),5-CF_3Ph	25% I at 1.0 μM

[a] Binding was performed using 0.3 nM [^{125}I]MCP-1 and human peripheral blood mononuclear cells at 23 °C (Ref. 5). IC_{50} values (*n*) are reported as mean ± SD (*n* = 2) or mean ± SEM (*n* > 2). For less potent compounds, % inhibition (% I) at a fixed inhibitor concentration is listed (*n* = 1).

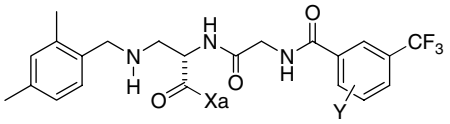
Given the positive effects of *ortho*- and *meta*-amine substitution, we examined N-substituted derivatives. As shown in Table 5, analogs with *ortho*-alkylamines, ureas, or carbamates are all potent antagonists (representative groups shown in **53**–**58**), but none are clearly superior to **50** in binding. The *meta*-amine can also be substituted with a variety of substituents (representative groups shown in **59**–**64**). Small groups are well-tolerated (see **59**, **60**), but large groups appear to reduce activity more sharply than in the *ortho* series (cf. **54**–**58** and **61**–**64**). Attempts to improve the affinity of each of the illustrated groups (alkyl, benzyl, urea, carbamate) in both the *ortho*- and *meta*-series have been unsuccessful (data not shown).

We studied the ability of our more potent compounds to antagonize the functional activity of MCP-1 on CCR2-bearing cells. As shown in Table 6, the potency of a given compound for inhibiting the MCP-1-induced Ca^{2+} flux can be readily predicted from the binding IC_{50}, but the activity in the monocyte chemotaxis assay cannot be as easily extrapolated. The installation of the *ortho*-amine functionality on the benzamide appears to enhance chemotaxis activity (cf. **2d** and **65**; also **2f** and **50**). Alkyl substitution on either the *ortho*- or *meta*-amino groups improves chemotaxis antagonism still further (cf. **50** and **53**, **54**, **57**; cf. **51** and **60**). Thus, simple blockade of chemokine binding is not sufficient to induce equipotent functional antagonism in the chemotaxis assay. Rather, specific structural features enable enhanced inhibition of cell migration. This phenomenon has been documented previously with a series of CCR3 antagonists.[13] Notably, the compounds do not induce Ca^{2+} flux when incubated with monocytes in the absence of MCP-1 (data not shown), indicating that they are antagonists, and not partial agonists. Additionally, the apparent IC_{50} for Ca^{2+} flux inhibition by **50** increases with increasing concentrations of MCP-1 (data not shown), indicating that this compound is a competitive antagonist of CCR2.

We also examined the selectivity profile of this series of CCR2 antagonists. None of the studied inhibitors exhibits significant binding to CCR1 (all <30% inhibition at 10 μM). These compounds also generally show high selectivity for binding to CCR2 relative to CCR3 (Table 6). The combination of *tert*-butylamide and *ortho*-aniline erodes this selectivity to ~100-fold when compared to the primary amide, substituted *ortho*-anilines, or the *meta*-aniline. We screened **50** against a broad panel of receptors, ion channels, and transporters (>100 targets). Compound **50** showed <30% inhibition at 0.1 μM against all of these targets. At higher concentrations (10 μM), it showed >70% inhibition of the histamine H2, sigma 1, CCR5, and opiate (mu) receptors, as well as the Na^+ and L-Ca^{2+} channels.

A number of chemokine receptor antagonists interact with a conserved glutamic acid in VII:06.[14] Indeed, others have shown that this residue is a key interaction site for four distinct CCR2 antagonist series, including the series represented by cyclic lead **8**.[14] In studies using a CCR2/3 chimera,[15] we have confirmed that **50** also

Table 6. Functional antagonism and chemokine receptor selectivity of the (*S*)-Dap–Gly inhibitors



Compound	Xa	Y	hCCR2 binding, IC_{50}[a] (nM)	Ca^{2+} Flux, IC_{50}[b] (nM)	Chemotaxis, IC_{50}[c] (nM)	hCCR3 binding, IC_{50}[d] (nM)
2d	NH_2	H	66 ± 5 (4)	69 ± 17	>1500 (n = 2)	>10,000
65	NH_2	2-NH_2	37 ± 8 (6)	82 ± 68	320	>10,000
2f	NH-*t*-Bu	H	48 ± 11 (13)	52 ± 30 (3)	>3000	>10,000
50	NH-*t*-Bu	2-NH_2	11 ± 4 (7)	8 ± 7	260 ± 20	750 ± 180 (3)
66[e]	NH-*t*-Bu	2-NH_2	20 ± 6 (6)	37 ± 21	150 ± 0	1,500 ± 370
53	NH-*t*-Bu	2-NHEt	13 ± 3 (4)	84	100 ± 50 (3)	>10,000
54	NH-*t*-Bu	2-NHBn	19 ± 7	57	39 ± 22	>10,000
57	NH-*t*-Bu	2-NHC(*O*)NH-*i*-Pr	15 ± 1	58	240	~10,000
51	NH-*t*-Bu	3-NH_2	23 ± 0	52	720 ± 130	>10,000
60	NH-*t*-Bu	3-NHEt	19 ± 8	58	190 ± 30	>10,000

Data are reported in one of three formats: result of a single determination; mean ± SD (n = 2, n not listed); or mean ± SEM (n indicated). Indication of an IC_{50} as ">" simply refers to <50% response at the indicated concentration (n = 1 unless otherwise indicated).

[a] Antagonism of binding of 0.3 nM [^{125}I]MCP-1 to human PBMCs at 23 °C (Ref. 5).

[b] Antagonism of Ca^{2+} flux induced by 10 nM MCP-1 in with THP-1 cells at 23 °C (Ref. 5).

[c] Antagonism of chemotaxis of human PBMCs induced by 10 nM MCP-1 at 37 °C (Ref. 5).

[d] Antagonism of binding of 0.15 nM [^{125}I]Eotaxin to CHO cells expressing human CCR3 (Ref. 13).

[e] 2-Me,4-Br–$PhCH_2$ replaces 2,4-Me_2PhCH_2.

capitalizes on this interaction: its binding to E291Q and E291A mutants was reduced by 470- and 345-fold, respectively, relative to binding to the wildtype.

In summary, we have discovered a new series of CCR2 antagonists. Modification of the scaffold through backbone methylation and alteration of spacing was not fruitful, whereas alteration of the terminal substituents provided for enhancements in potency. The most active compounds exhibit binding affinities <50 nM, and are antagonists of both Ca^{2+} and chemotaxis responses induced by MCP-1. These compounds also show good selectivity for binding to CCR2 relative to CCR1 and CCR3. Our hypothesis is that these compounds are acting as acyclic mimics of the lead **8**, as judged by the SAR (Table 1 and Fig. 2), computational modeling (Supporting Information), and receptor mutagenesis (see above). Importantly, these compounds introduce an additional site for modification to tune selectivity and/or potency. The exploitation of this observation will be the focus of future publications.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.07.028.

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