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Diaryl substituted pyrazoles as potent CCR2 receptor antagonists

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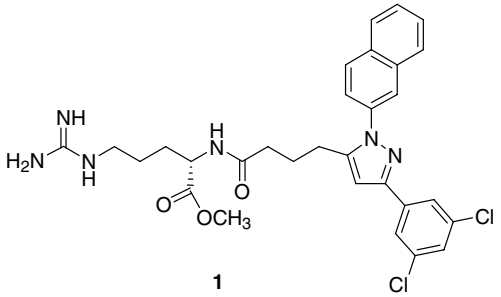
Abstract—We have identified and synthesized a series of diaryl substituted pyrazoles as potent antagonists of the chemokine receptor subtype 2. Structure–activity relationship studies directed toward improving the potency led to the discovery of **23** (IC_{50} = 6 nM).

Chemokines[1] (*chemo*tactic cyto*kines*) are a class of small molecular weight peptides that are released by a wide variety of cells to attract cells such as monocytes, macrophages, T cells, eosinophils, basophils, and neutrophils to sites of inflammation.[2] In particular, monocyte chemoattractant protein-1 (MCP-1), which is included within the CC class of chemokines, is a key monocyte chemoattractant[3] acting primarily through its receptor, CCR2,[4] which is a member of the G-protein coupled receptor (GPCR) superfamily.[5] The critical role of MCP-1 and CCR2 in monocyte migration to sites of inflammation has been highlighted by the MCP-1[6] and CCR2[7] knockout mice. Numerous studies have implicated the importance of MCP-1 and CCR2b in a variety of inflammatory diseases,[8] including rheumatoid arthritis,[9] atherosclerosis,[10] multiple sclerosis,[11] glomerulonephritis[12] and stroke.[13] Therefore, the therapeutic potential of CCR2 antagonists in treating a wide range of inflammatory diseases has attracted considerable interest, including the disclosure in the literature of a number of small molecule CCR2 antagonists.[14–23] This paper outlines the discovery and optimization of a new class of CCR2 antagonists based on a diarylpyrazole core which originated from screening of the Merck sample collection. This screen identified diarylpyrazole **1** as a potential lead with a CCR2 IC_{50} of 221 nM. Our goal then became to optimize the potency of this lead structure.



1

The compounds described herein were synthesized as outlined in Schemes 1–4.[24] For the diarylpyrazoles with a carbon linkage, synthesis began with reaction of a ketoester (**2**) and a hydrazine (**3**) in neat acetic acid to form cyclic products (**4**) in moderate yields (Scheme 1). These were converted to bromopyrazoles **5**

Keywords: Chemokines; CCR2; MCP-1; GPCR.
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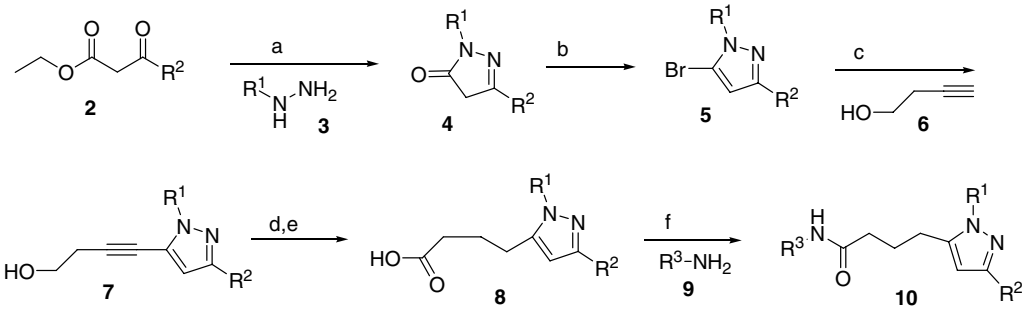
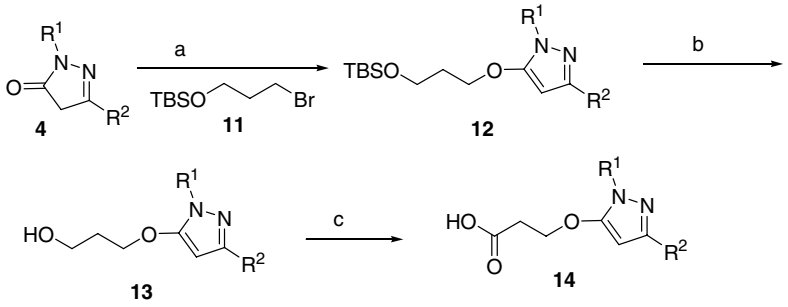
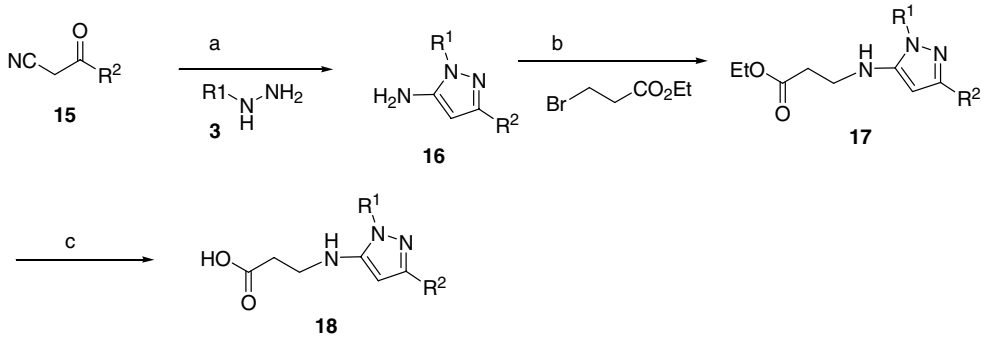


Scheme 1. Reagents and conditions: (a) HOAc (neat), 130 °C, 40–65%; (b) PBr_3, CH_3CN, microwave, 150 °C, quant; (c) $Pd(PPh_3)_4$, CuBr, Et_3N, 70 °C, 65–85%; (d) Pd/C, H_2, EtOAc, rt, 70–90%; (e) PDC, DMF, rt, 55–80%; (f) EDCI, DMAP, CH_2Cl_2, rt, 75–95%.



Scheme 2. Reagents and conditions: (a) K_2CO_3, DMF, 60 °C, 85–95%; (b) TBAF, THF, rt, quant; (c) PDC, DMF, rt, 65–70%.



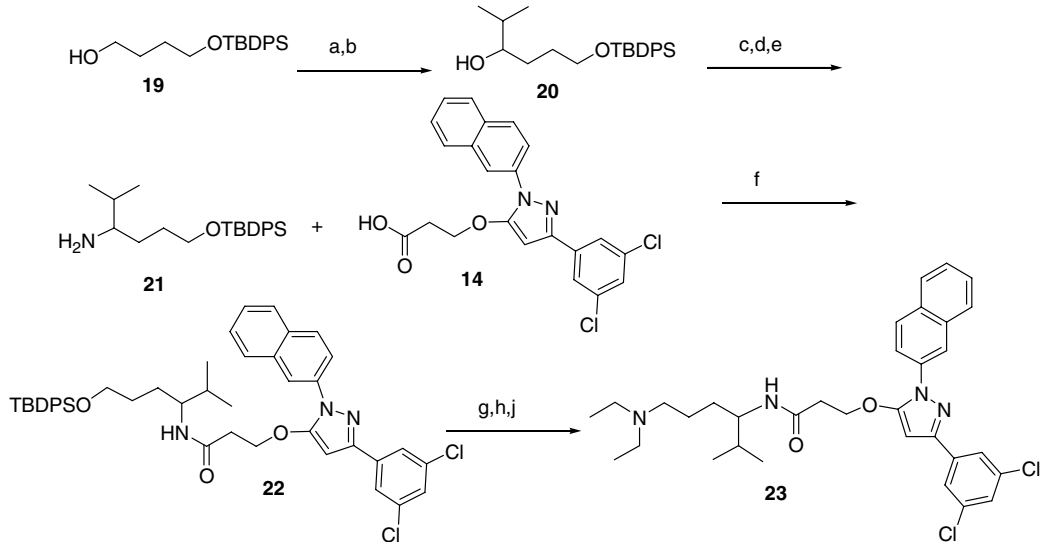
Scheme 3. Reagents and conditions: (a) $PhCH_3$, microwave, 130 °C, 85–95%; (b) K_2CO_3, NaI, DMF, microwave, 200 °C, 65–72%; (c) LiOH, MeOH/THF 9/1, 70 °C, quant.

via reaction with phosphorous tribromide under microwave irradiation in quantitative yields. Bromopyrazoles **5** were then coupled to 3-butyn-1-ol (**6**) under Sonagashira conditions to give alkyne-pyrazoles **7**. Reduction of the triple bond followed by oxidation of the pendant alcohol gave acids **8**, which were then coupled with the appropriate amines (**9**) under standard conditions to give the desired compounds. In this manner the compounds in Tables 1 and 4 were synthesized.

For the diarylpyrazoles with an oxygen linker, synthesis began with the same intermediate (**4**) as described in Scheme 1. These compounds were alkylated with bromide **11** to give oxopyrazoles **12** in good yields (Scheme 2). After removal of the protecting group, oxidation gave acids **14**, which were reacted as above using standard coupling conditions to give the desired compounds. Compounds in Tables 2 and 3 were synthesized in this fashion.

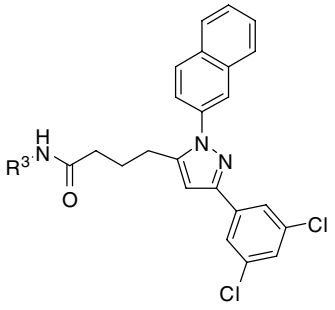
For nitrogen substituted diarylpyrazoles, synthesis began with cyanoketones **15** and hydrazines **3** (Scheme 3). Reaction under microwave irradiation gave aminopyrazoles **16** in excellent yields. Alkylation with 3-bromoethylpropionate gave esters **17**, which were hydrolyzed to give acids **18** and further reacted as described above to give the desired compounds. Compounds **35** and **37** were synthesized via this scheme.

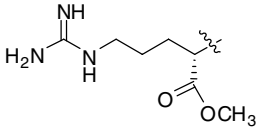
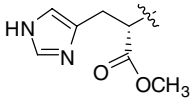
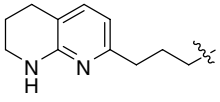
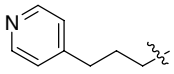
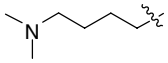
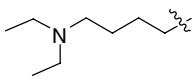
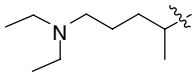
Analogs **23** and **39**–**43** were synthesized in the fashion shown in Scheme 4. Oxidation and addition of isopropylmagnesium bromide to monoprotected 1,4-butanediol (**19**) gave alcohol **20** which was converted to amine **21** in good yield using a three-step procedure (tosylation, displacement with sodium azide, and reduction). Coupling of **21** with acid **14** (Scheme 2) gave pyrazole **22**. Deprotection, followed by oxidation of the primary alcohol, gave an aldehyde which was reductively



Scheme 4. Reagents and conditions: (a) TPAP, NMO, 4 Åmol sieves, CH_2Cl_2/CH_3CN 3/1, rt, quant; (b) *i*-PrMgBr, THF, 0 °C, 72%; (c) Ts-Cl, pyridine, 0 °C; (d) NaN_3, DMF, 40 °C; (e) Pd/C, H_2, MeOH/EtOAc 1/1, rt, 70% over 3 steps; (f) EDCI, DMAP, DMF, 40 °C, 65%; (g) TBAF, THF, rt, quant; (h) Dess–Martin, CH_2Cl_2, rt; (j) diethylamine, $NaBH_3CN$, AcOH, THF, rt, 55% over 2 steps.

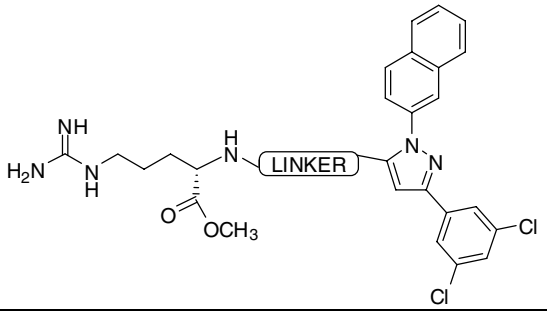
Table 1. Effect of pendant amide group on binding affinity

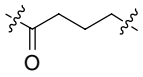
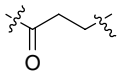
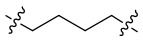
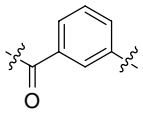
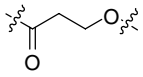
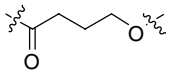
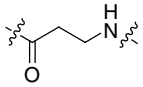
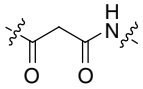


Compound	R^3	CCR2 IC_{50}[a] (nM)	Chemotaxis IC_{50} (nM)
1		221	185
24		2840	NT[b]
25		9332	NT[b]
26		1123	1400
27		1803	NT[b]
28		1225	NT[b]
29		108	209

[a] Value represents mean of two or more experiments. The standard deviation limits are within 25% of the reported values.
[b] NT, not tested.

Table 2. Linker modifications



Compound	LINKER	CCR2 IC_{50} (nM)[a]	Chemotaxis IC_{50} (nM)
1		221	185
30		4741	NT[c]
31		NA[b]	NT[c]
32		NA[b]	NT[c]
33		62	118
34		7601	NT[c]
35		43	68
36		NA[b]	NT[c]

[a] Value represents mean of two or more experiments. The standard deviation limits are within 25% of the reported values.
[b] NA denotes not active <10 μM concentration.
[c] NT, not tested.

aminated with diethylamine to give analog **23** in moderate yield. Compounds **39**–**43** were synthesized in the same fashion using the appropriate Grignard reagent.

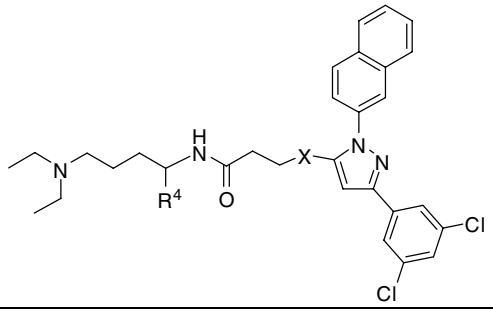
In order to investigate the SAR and improve the potency of compound **1** four areas were addressed: (1) replacement of the arginine of the lead compound with a variety of other amino acids as well as simple amines, (2) investigation of the linker between the diarylpyrazole moiety and the amine group, (3) substitution on a potent diethylamine scaffold, and (4) examination of other aryl groups around the pyrazole. All compounds described herein were synthesized and tested as racemates unless otherwise noted. Two in vitro assays were used to drive the SAR. The primary assay monitored inhibition of [^{125}I]MCP-1 binding to primary human blood monocytes which endogenously express CCR2.[25] Second, a functional chemotaxis assay that detected the ability to inhibit the MCP-1 induced migration of monocytes was used.[26]

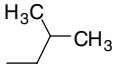
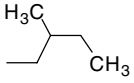
We initially examined the replacement of the arginine group from the lead structure (Table 1). A number of other amino acid esters were incorporated, but only histidine **24** was found to be moderately active with an IC_{50} of 2840 nM. Other amino acid esters such as glycine, alanine, phenylalanine, tyrosine, and glutamate methyl ester all gave inactive compounds, as did the enantiomer of compound **1** (data not shown). We then examined a large number of other amine containing side chains, mainly focusing on accessing the same binding site as the guanidine group. Most of these attempts led to inactive compounds, although analog **25** did have weak activity at 9332 nM. We then turned to a number of simpler amine containing side chains. Some success was achieved in this area with pyridine **26** and dimethylamine **27** with IC_{50}s of 1123 and 1803 nM, respectively. Extending the alkyl chain to a diethyl amino group as in **28** slightly improved the activity (simple cyclic amines such as pyrrolidine and piperidine in this position were less active—data not shown). A large boost in potency was observed when a methyl group was incorporated α to the amide to give compound **29** with an IC_{50} of 108 nM as well as good activity in the chemotaxis assay (IC_{50} = 209 nM). This result points to the importance of a group α to the amide to give potent compounds in this series, an effect that was examined further (vide infra).

We next turned to the effect of the linker between the diaryl pyrazole group and the arginine side chain on activity (Table 2). It appears that the SAR is relatively tight for modifications in this area. For example, shortening the chain one carbon, as in **30**, leads to a precipitous drop in activity to 4741 nM. Analog **31** highlights the importance of the central amide for potency—removal of the carbonyl gives a compound that is inactive. Likewise, constraining the linker as in phenyl analog **32** gives an inactive compound. Better results were achieved when an additional heteroatom was incorporated next to the pyrazole. Both oxygen analog **33** and nitrogen analog **35** gave a 4- to 5-fold boost over the original lead with IC_{50}s of 62 and 43 nM, respectively. Furthermore, these analogs were also more potent in the functional chemotaxis assay (IC_{50} = 118 nM for **33** and 68 nM for **35**). Further modifications were not as well tolerated. Extending analog **33** by one atom to **34** leads to over a 100-fold drop in potency. Likewise, incorporating an additional amide as in analog **36** gave an inactive compound.

A combination of the diethylamino side chain with various linker combinations was then explored (Table 3).[27] Incorporation of nitrogen (**37**) or oxygen (**38**) led to a boost in potency as was observed with the arginine analogs. We then focused on a series of oxygen linked compounds wherein the group α to the amide (R^4) was varied. Increasing the size of the group from methyl to ethyl (**39**) led to a large boost in potency both in the binding (IC_{50} = 9 nM) and functional (IC_{50} = 83 nM) assays. Increasing the size to an *n*-propyl (**40**) group gave a compound that was similar in potency to methyl

Table 3. Dialkylamino containing pyrazoles

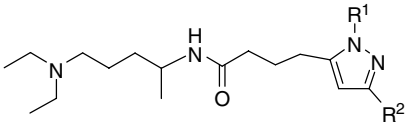


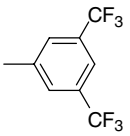
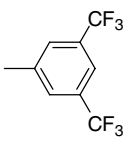
Compound	R^4	X	CCR2 IC_{50}[a] (nM)	Chemotaxis IC_{50} (nM)
29	$-CH_3$	$-CH_2-$	108	209
37	$-CH_3$	–NH–	30	54
38	$-CH_3$	–O–	66	280
39	$-CH_2CH_3$	–O–	9	83
40	$-CH_2CH_2CH_3$	–O–	44	154
23		–O–	6	32
41		–O–	74	178
42		–O–	107	225
43	$-CH_2(CH_2)_2CH_3$	–O–	52 at 1 μM	NT[b]

[a] Value represents mean of two or more experiments. The standard deviation limits are within 25% of the reported values.
[b] NT, not tested.

analog **38** (IC_{50} = 44 nM vs 66 nM). An isopropyl group (**23**) appeared to be the optimal substituent in this series, with an IC_{50} of 6 nM in the binding assay and an IC_{50} of 32 nM in the chemotaxis assay. This compound represents one of the more potent CCR2 antagonists reported to date. Increasing the size

Table 4. Pyrazole substitution



Compound	R^1	R^2	CCR2 IC_{50}[a] (nM)	Chemotaxis IC_{50} (nM)
29	2-Naphthyl	3,5-Dichlorophenyl	108	209
44	2-Naphthyl	2-Chlorophenyl	69% at 10 μM	NT[b]
45	2-Naphthyl	3-Chlorophenyl	168	57 at 300 nM
46	2-Naphthyl	4-Chlorophenyl	442	NA at 300 nM
47	2-Naphthyl	3-Methoxyphenyl	2391	NT[b]
48	2-Naphthyl	3-Fluoro phenyl	522	NT[b]
49	2-Naphthyl	3-Trifluoromethylphenyl	207	NT[b]
50	2-Naphthyl		634	NT[b]
51	Phenyl	3,5-Dichlorophenyl	69% at 10 μM	NT[b]
52		3,5-Dichlorophenyl	6475	NT[b]

[a] Value represents mean of two or more experiments. The standard deviation limits are within 25% of the reported values.
[b] NT, not tested.

Table 5. Rat pharmacokinetic parameters for selected compounds

Compound	Cl[a] (mL/min/kg)	V_d (L/kg)	$t_{1/2}$ (h)	%F[a]
1	8.3	0.98	2.49	0
29	28.1	13.0	6.52	5

[a] Dosed 1 mpk iv (n = 2) and 2 mpk po (n = 3) in Sprague–Dawley rats.

beyond isopropyl led to a rapid drop off in potency, as seen in analogs **41**–**43**.

Lastly, we investigated the effect of different aryl substituents around the pyrazole ring. In general, modifications of the original scaffold (**29**) led to compounds with considerably lower activity.[27] Monochloro analogs at R^2 were examined first, with only the 3-chlorophenyl compound **45** giving binding activity comparable to **29** (68 nM vs 108 nM), indicating that at least one substituent at the 3 position was optimal for good activity. Other 3-substituted aryl groups were examined, including methoxy (**47**), fluoro (**48**), and trifluoromethyl (**49**), but in general activity was diminished. Finally, a trifluoromethyl analog (**50**) of compound **29** gave 6-fold diminished potency compared to the lead. Thus, the 3,5-dichlorophenyl moiety appeared to be the optimal group. Attempts to replace the 2-naphthyl group at R^1 were less successful, with both a phenyl (**51**) and a bistrifluoromethyl (**52**) group giving dramatically lower activity.

Two of the diarylpyrazoles described above were examined for their pharmacokinetic properties in rat. As shown in Table 5, compound **1** has moderate rat PK following iv dosing with low clearance, albeit with no oral bioavailability. Compound **29** had a somewhat higher clearance but slightly better oral bioavailability.

Selectivity of compounds for CCR2 over the most closely related CC chemokine receptors, CCR1 and CCR5, was determined. All the compounds described above were selective against CCR1. CCR2 and CCR5 have a higher degree of sequence homology (71% identity between CCR2b and CCR5),[28] and therefore separation of activity at these two receptors might be anticipated to be more difficult. For the series described here, selectivity could be achieved for some compounds. For example, although compounds **1** (CCR2 IC_{50} = 221 nM, CCR5 IC_{50} = 63 nM) and **29** (CCR2 IC_{50} = 108 nM, CCR5 IC_{50} = 220 nM) were comparable in activity, compound **23** had considerable selectivity for CCR2 (CCR2 IC_{50} = 6 nM, CCR5 IC_{50} = 1610 nM).

In conclusion, a new class of diaryl pyrazole containing compounds that are potent antagonists at the CCR2 receptor has been developed from a screening lead. These compounds are among the most potent CCR2 antagonists yet described and should be useful pharmacological tools to further elucidate the role of CCR2 in inflammatory diseases.

Acknowledgment

We thank Merryl Cramer for assistance in measuring the PK parameters for **1** and **29**.

References and notes

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