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N,*N*′-Diarylcyanoguanidines as antagonists of the CXCR2 and CXCR1 chemokine receptors

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Abstract—A series of *N*-(2-hydroxy-3-sulfonamidobenzene)-*N*′-arylcyanoguanidines was prepared. In general, these compounds proved to be potent antagonists of CXCR2 while the selectivity versus CXCR1 ranged from non-selective to >200-fold.
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Interleukin-8 (IL-8, CXCL8) and related CXC chemokines (ENA-78 (CXCL5), GCP-2 (CXCL6), GROα (CXCL1), GROβ (CXCL2), and GROγ (CXCL3)) play an important role in the trafficking of immune cells to sites of inflammation which is consistent with their potential involvement in pathophysiological processes such as arthritis, reperfusion injury, and asthma. Indeed, elevated plasma levels of IL-8 and GROα have been associated with these conditions in humans.[1] Thus far, two seven-transmembrane G-protein coupled receptors have been identified, which are activated by IL-8 (CXCR1 and CXCR2). CXCR1 binds IL-8 and GCP-2 with high affinity, while CXCR2 binds several ELR+ chemokines including IL-8, GCP-2, ENA-78, GROα, GROβ, and GROγ with high affinity.[2] The potential therapeutic value for small-molecule antagonists of the IL-8 receptors is further supported by studies done with CXCR2 mouse gene knockouts which show elevated leukocytes and lymphocytes without apparent pathogenic consequences indicating that these receptors are not required for normal physiology.[3]

As previously disclosed,[4–6] a series of *N*,*N*′-diarylureas has been identified as potent, selective CXCR2 antagonists. This series was later expanded to include *N*,*N*′-diarylguanidines[7] (Fig. 1).

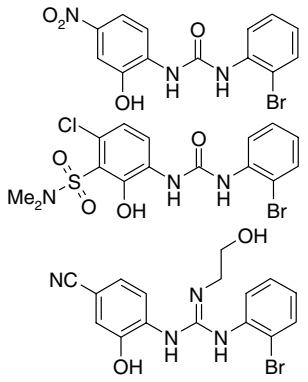

Figure 1. Examples of urea- and guanidine-type CXCR2 antagonists.

As a natural extension of the work with *N*,*N*′-diarylguanidines, it was decided to examine guanidine-like urea isosters. A series of *N*,*N*′-diarylcyanoguanidines was prepared as part of this study. The syntheses started from the sulfonyl chlorides **1** which were prepared in high yields according to a previously published procedure.[4,8] These were reacted with a variety of amines yielding the sulfonamides **2** which upon hydrolysis of the oxazole moiety gave the aminophenols **3**. Treatment with thioisocyanates resulted in the formation of the thioureas **4**. Protection of the phenolic function as the TBS ether followed by exposure to methanesulfonyl chloride in the presence of triethylamine yielded the carbodiimides **5**.[9] Addition of cyanamide followed by TBS ether cleavage produced the target compounds **6** (Scheme 1).

Keywords: CXCR2; CXCR1; Cyanoguanidine.
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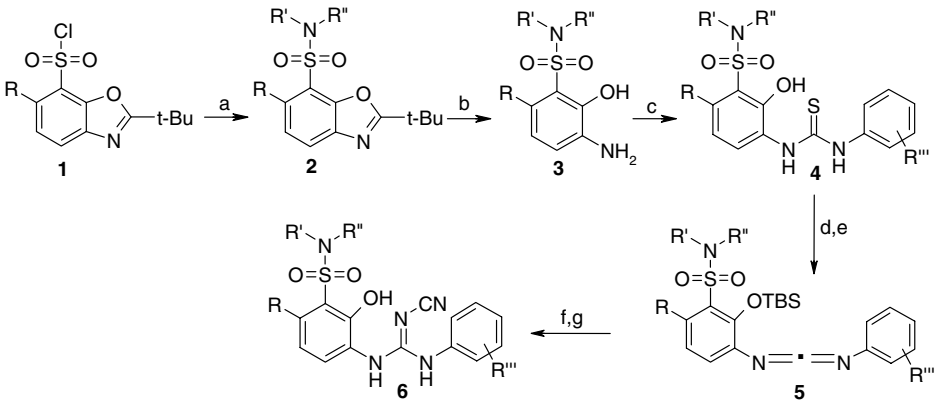


Scheme 1. Preparation of *N*,*N*′-diarylcyanoguanidines. Reagents and conditions: (a) R′R″NH, Et_3N, CH_2Cl_2, rt; (b) 10% H_2SO_4, reflux; (c) ArN=C=S, DMF, rt; (d) TBSCl, imidazole, CH_2Cl_2, rt; (e) MsCl, Et_3N, rt; (f) NH_2CN, $(i\text{-Pr})_2NEt$, rt; (g) CsF, MeOH, 0 °C.

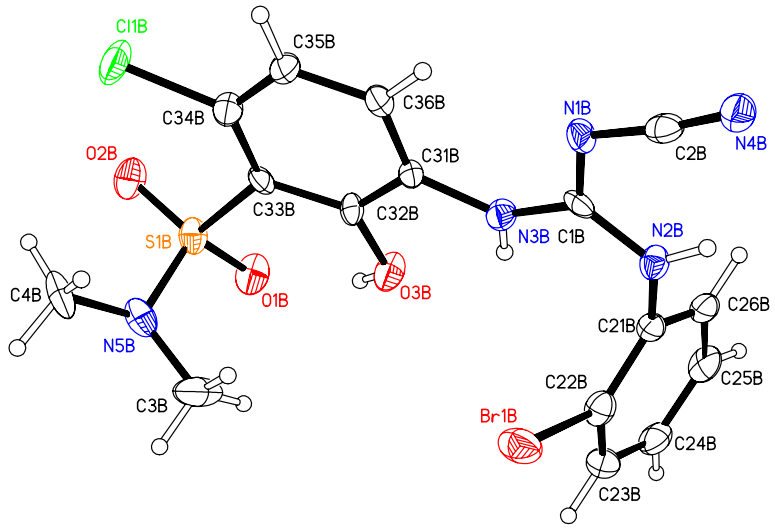


Figure 2. X-ray crystal structure of **6c** (CCDC 616833).

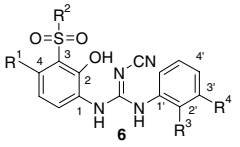
Single crystal X-ray crystallographic analysis of **6c** showed that the cyano group was situated trans to the phenol-bearing aniline moiety (Fig. 2).[10]

The compounds **6** were prepared under the assumption that the structure activity relationships (SAR) which had been observed in the urea series relative to CXCR2 would translate into the cyanoguanidine series. Therefore, the acidic phenol function at the 2-position was retained because it had been found to be essential for high receptor affinity and the 5-, 6-, 4′-, 5′-, and 6′-positions as well as the cyanoguanidine nitrogens were left unsubstituted since substitution at these positions had been found to reduce receptor affinity.[5,11] Also, the 3-sulfonamido group was incorporated as it has been demonstrated to enhance bioavailability by reducing the rate of glucuronidation of the adjacent phenol.[6] This assumption largely held true and allowed us to focus on examining the SAR related to the 4-, 1-′, 2′-, and 3′-positions as well as the substitution on the sulfonamide. One surprising finding was that a number of these compounds displayed substantially lower selectivity versus the CXCR1 receptor relative to what had been observed with previous compound series. This allowed us to gather important pharmacophore information on this receptor as well and determine similarities and differences between the binding sites of two receptors. Our findings indicate that a range of small substituents are tolerated at the 4-position: Neither receptor shows a great deal of discrimination between chloro, fluoro, methyl, trifluoromethyl or hydrogen. It appears that the electronic nature of the substituent has little effect on the binding affinity for either receptor. In the urea series, the degree of phenol-ionization at neutral pH has been found to significantly influence the affinity for the CXCR2 receptor.[11] That may be the case in this series as well since it appears that in the presence of the sulfonamide, the substituent at the 4-position has surprisingly little influence on the pK_a of the phenol. Thus, the 4-chloro compound **6l** has a pK_a of 6.41, while the similar 4-hydro compound **6r** has a pK_a of 6.48. As for the substitution on the sulfonamide, a clear difference between the receptors was observed: While the introduction of a carboxylic acid moiety had little influence on the CXCR2 affinity, the CXCR1 affinity was increased considerably, thus yielding a potent dual

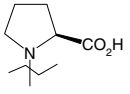
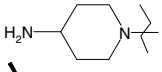
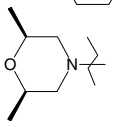
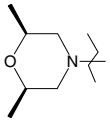
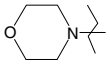
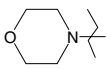
receptor antagonist (**6j**). The presence of other types of hydrogen bond donors or acceptors as well as steric bulk did not seem to affect either receptor greatly. The other clear difference between the receptors was observed at the 2′-position. Here, CXCR1 displayed different steric and perhaps electronic requirements than CXCR2. The CXCR2 receptor tolerated quite large aromatic substituents (such as **6d** and **6e**), while the bulkier isopropyl substituent (**6g**) substantially reduced the activity. The electronic nature of the 2′-substituent seemed to be inconsequential to CXCR2, while the requirements for CXCR1 were less obvious. Bromine appeared to be optimal for CXCR1, while larger substituents as well as fluorine resulted in sharply reduced affinities. Substitution at the 3′- and especially the 4′-position negatively affected the affinity for both receptors. These findings suggest that the yet to be identified small molecule binding regions[13] of the CXCR2 and CXCR1 receptors are relatively similar in structure, but that the CXCR1 receptor is relatively restrictive, whereas the CXCR2 receptor can accommodate a wider range of pharmacophors (Table 1). This is further illustrated by the fact that no CXCR1 selective and IL-8 competitive antagonist has been described to date, while a substantial number of structurally distinct, selective CXCR2 antagonists have been reported.[14]

In conclusion, a series of *N*,*N*′-diarylcyanoguanidines were prepared and evaluated as antagonists of CXCR1 and CXCR2. The majority of the prepared compounds were potent CXCR2 antagonists while the selectivity relative to CXCR1 ranged from non-selective (**6j**) to >200-fold (**6i**). The discovery of potent dual antagonists is a promising development which may lead to novel

Table 1. Affinities of *N*,*N*′-diarylcyanoguanidines for CXCR1 and CXCR2[a]



6

Compound **6**	R^1	R^2	R^3	R^4	CXCR2[b] (IC_{50}, nM)	CXCR1[b] (IC_{50}, nM)
a	Cl	NMe_2	Me	H	17	378
b	Cl	NMe_2	F	H	9	1548
c	Cl	NMe_2	Br	H	5	55
d	Cl	NMe_2	Obn	H	9	293
e	Cl	NMe_2	Ph	H	13	752
f	Cl	NMe_2	Et	H	15	512
g	Cl	NMe_2	*i*-Pr	H	112	7289
h	Cl	NMe_2	CF_3	H	12	411
i	Cl	NMe_2	F	Cl	29	6204
j	Cl		Br	H	16	22
k	Cl		Br	H	22	188
l	Cl		Br	H	9	215
m	Cl		Me	H	17	1082
n	Me	NMe_2	Br	H	7	187
o	Me		Br	H	6	104
p	F	NMe_2	Br	H	4	192
q	CF_3	NMe_2	Br	H	7	100
r	H		Br	H	12	582

[a] Binding assays were performed on Chinese hamster ovary (CHO) cell lines expressing either CXCR1 or CXCR2. The CHO-CXCR1 and CHO-CXCR2 membranes were prepared according to Kraft and Anderson.[12] All assays were performed in 96-well microtiter plates using radiolabeled [^{125}I]IL-8 (human recombinant, concn: 0.23 nM). The binding results are expressed as a mean of three individual experiments.

[b] The functional activities of compounds **6c**, **6d**, and **6l** were tested in a calcium mobilization assay performed with human neutrophils using GROα as the ligand. The resulting IC_{50}s were within 5-fold of the binding IC_{50}s reported in this table.

therapeutic agents that effectively block all aspects of ELR+ chemokine induced inflammatory responses. In addition, these compounds may be useful for the further elucidation of CXCR1 pharmacology.[15]

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