Evaluation of Potent and Selective Small-Molecule Antagonists for the CXCR2 Chemokine Receptor

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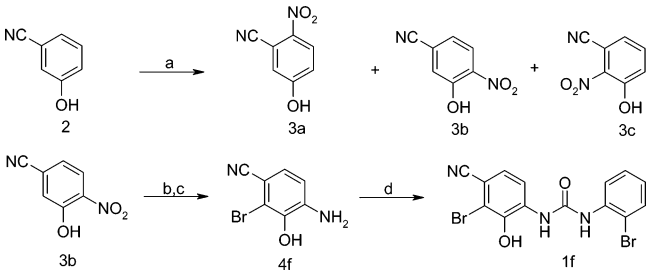
Abstract: *N*,*N*′-Diarylureas were prepared, and the structure−activity relationship relative to the CXCR2 receptor was examined. This led to the identification of a potent and highly selective CXCR2 antagonist, which in addition was shown to be functionally active both in vitro against human neutrophils and in vivo in rabbit models of ear edema and neutropenia.

Interleukin-8 (IL-8) and related CXC chemokines (ENA-78, GCP-2, GROα, *β* and *γ*) play an important role in the trafficking of neutrophils that 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 and chemokines including IL-8, GCP-2, ENA-78, GROα, GRO*β*, and GRO*γ* with high affinity.[2] The potential therapeutic value for small-molecule antagonists of the IL-8 receptor(s) is further supported by studies done with CXCR2 mouse gene knockouts that show elevated lymphocytes without apparent pathogenic consequences, indicating that these receptors are not required for normal physiology.[3] Herein, we disclose the design and synthesis of a series of potent and selective antagonists of CXCR2, a representative of which is *N*-(3-bromo-4-cyano-2-hydroxyphenyl)-*N*′-(2-bromophenyl)urea (**1f**).[4]

Chemistry. The synthesis of the most potent urea antagonist, **1f**, starting from the commercially available 3-cyanophenol, is shown in Scheme 1. Thus, the 3-cyanophenol **2** was nitrated with $NaNO_3$ and sulfuric acid in a biphasic solution of methylene chloride and water. Although the nitration was not selective and, indeed, the para nitro compound predominates, this route was attractive because of the large number of commercially available phenols. The ortho nitrophenol **3b** can easily be separated by flash chromatography. Selective bromination of the ortho position **3b** was achieved using hexamethylenediamine bromide perbromide, and the 2-bromo-3-cyano-6-nitrophenol was then reduced using tin(II) chloride under acidic conditions to provide the ortho aminophenol **4**. Compound **4** was subsequently coupled with 2-bromophenyl isocyanate to form the urea **1f**. Compounds **1d** and **1e** were made by the reduction of **3b** using palladium on barium sulfate under a hydrogen atmosphere, followed by the coupling of the product aniline with the requisite isocyanate. Compound **1g** was synthesized from the corresponding 2-chloro-3-cyanophenol obtained from **3c** by reduction of the nitro group using hydrogen gas with 10% Pd/C as a catalyst, followed by a Sandmeyer reaction (*tert*-butyl nitrite, $CuCl_2$). Urea **1h** was synthesized from the 3-chloro-2-cyanophenol, which was obtained from the commercially available 2-benzyloxy-6-chlorobenzonitrile upon treatment with trifluoroacetic acid. The 2-chloro 3-cyanophenol and the 3-chloro-2-cyanophenol were converted to ureas **1g** and **1h** according to the route shown in Scheme 1. Urea **1b** was synthesized by the monoreduction of 2,6-dinitrophenol with dicyclohexene and catalytic Pd/C. The resulting aniline was coupled with phenyl isocyanate to form **1b**. The syntheses of **1a** and **1c** were accomplished by coupling the commercially available nitro-2-amino phenols with phenyl isocyanate.

Scheme 1. Synthesis of CXCR2 Antagonist **1f**[a]



[a] Reaction conditions: (a) $NaNO_2$, $NaNO_3$, H_2SO_4, CH_2Cl_2, H_2O, 23 °C; (b) HMDA·Br_2·HBr, CH_2Cl_2, 23 °C, 30%; (c) $SnCl_2$, EtOH, 85%; (d) 2-BrPhNCO, DMF, 32%.

Results and Discussion. High-throughput screening of our compound collection utilizing a membrane binding assay expressing CXCR2 led to the identification of urea **1a**. This compound competed with [^{125}I]-IL-8 in a binding assay using membranes of Chinese hamster ovary (CHO) cells stably expressing CXCR2 (IC_{50} = 330 nM), whereas it failed to compete up to 33 *μ*M in the corresponding assay using membranes expressing CXCR1.[5,6] In addition, urea **1a** was shown to be a competitive, functional antagonist of GROα-induced calcium influx in human polymorphonuclear neutrophils (PMNs) with an IC_{50} of 340 nM. GROα is a selective agonist only activating CXCR2 in these cells. Compound **1a**'s ability to block IL-8-induced calcium mobilization in these cells was considerably diminished (IC_{50} = 25 *μ*M), since IL-8 induces calcium mobilization through both CXCR1 and CXCR2, the former receptor being unaffected by **1a**. Because of the potency and high selectivity of **1a** (Table 1) for the CXCR2, an SAR evaluation of this lead was undertaken. Initial synthetic studies focused on determining the importance of the acidity of the phenol for the activity of this compound. Evaluation of **1a** inhibition at CXCR2 as a function of pH indicated that this compound was more potent in the anionic form.[7] However, the lower potency of the ortho and para nitro analogues **1b** and **1c** revealed that

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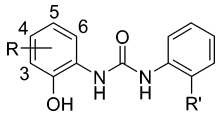
Table 1. Effect of Compounds **1a**−**h** on Inhibition of Binding of IL-8 to Membranes of Cloned CXCR1 and CXCR2 in CHO Cells, GROα-Mediated Calcium Mobilization in Human PMNs, and DTH in Rabbits



compd	R	R′	IC_{50} of CXCR2 (nM)[a]	IC_{50} of CXCR1 (nM)[a]	ratio R1/R2	IC_{50} of GROα Ca^{2+} flux (nM)[b]	DTH, % inhibition (dose (μg))[c]
1a	4-NO_2	H	320 ± 60	18200 ± 1500	60	339 ± 102	
1b	3-NO_2	H	860 ± 200	33200 ± 3000	39	511 ± 241	
1c	5-NO_2	H	10900 ± 460	46200 ± 730	4.2	13,760 ± 5,740	
1d	4-CN	H	200 ± 30	21000 ± 3000	105	120 ± 35	21 (1000)[d]
1e	4-CN	2-Br	25 ± 5	15100 ± 650	600	53 ± 29	31 (500)
1f	3-Br, 4-CN	2-Br	6 ± 2	2600 ± 500	433	0.79 ± 0.17	33 (500)
1g	3-Cl, 4-CN	2-Br	22 ± 1	4200 ± 900	190	2.6 ± 0.7	
1h	3-CN, 4-Cl	2-Br	57 ± 3	11100 ± 600	194	7.5 ± 2.0	3 (62.5)[d,e]

[a] Binding assays were performed on CHO cell lines expressing either CXCR1 or CXCR2. The CHO-CXCR1 and CHO-CXCR2 membranes were prepared according to Kraft and Anderson.[10] All assays were performed in 96-well microtiter plates using radiolabeled [^{125}I]-IL-8 (human recombinant).[4] The binding results are expressed as a mean of three individual experiments ± SEM. [b] The calcium mobilization assay was performed with human neutrophils using GROα as a CXCR2 selective ligand. The results are expressed as the mean ± SEM of at least three separate determinations. [c] The DTH assay was conducted in rabbits. The animals were sensitized by the injection of complete Freund's adjuvant in the nape of the neck. Five weeks later, the animals were challenged intradermally with tuberculin (10 units purified protein derivative (PPD)). Five minutes after challenge, methanol (vehicle) or test compound (50 μL, methanol solution) was applied to the PPD sites. Vehicle or test compound solution was reapplied 4 h later, and the DTH response was measured 48 h after PPD challenge. The response was measured by determining the thickness of the skin fold in the area of the PPD challenge. Control response varied from 86.8 ± 7.0 to 127.1 ± 10.3 cm^{-3} (mean ± SEM). [d] The observed response was not statistically significant. [e] The dose was limited by the solubility of the test compounds in methanol.

the acidity of the phenol is not the only determinant of activity. Our early investigation indicated that 4-substitution was preferred over 5-substitution; this latter substitution was generally detrimental to activity.[6] Substitution at the 3-position, ortho to the phenol, is well tolerated although the 4-position substitution is generally favored. After the optimal placement of the nitro group on the phenolic ring was determined, attention was focused on replacement of this group because of concerns related to the possible long-term toxicity. In general, more electron-withdrawing substituents were preferable as a result of their enhancement of the acidity of the phenol. A survey of electron-withdrawing groups at the 3- and 4-position determined that a cyano at the 4-position was a viable replacement for the nitro group. Thus, **1d** has increased affinity for CXCR2 with an IC_{50} of 200 nM (cf. IC_{50} = 320 nM for the corresponding nitro compound **1a**). Evaluation of the SAR around the other phenyl ring indicated that substitution at the 2-position improved activity while substitutions at either the 3,4,5-position or 2,6-disubstitutions were generally detrimental to activity.[6] The 2-bromo substituent (**1e**−**h**) appeared to have the greatest effect, increasing the affinity for CXCR2 nearly 10-fold while having little effect on the affinity for CXCR1. By use of the optimum 2-bromo substituent in the non-phenolic ring, disubstitution at the 3- and 4-position of the phenolic ring was then examined. This effort yielded the highest affinity compound of the series, *N*-(3-bromo-4-cyano-2-hydroxyphenyl)-*N*′-(2-bromophenyl)urea (**1f**), which had an IC_{50} of 6 nM. The nature of substituents at the 3-position appears to be quite important because a fairly subtle change from a bromine to a chlorine results in a significant decrease in activity (3-fold). The data that have currently been generated with compounds in this series appear to be consistent with a two-point binding model where a hydrogen bond donor of the receptor interacts with the substituent at either the 3- or 4-position and another H-bond donor interacts with the phenolic anion. The *N*-(3-bromo-4-cyano-2-hydroxyphenyl)-*N*′-(2-bromophenyl)urea **1f** is a potent, selective CXCR2 antagonist showing no activity in 13 other receptor binding assays. Scatchard analysis revealed that this compound is a competitive inhibitor of CXCR2 in the GROα-mediated calcium mobilization assay in PMNs with a K_b of 0.42 nM (Figure 2). Phenols **1a**−**h** have been used as tool compounds to evaluate the role of CXCR2 both in vitro and in vivo. Thus, *N*-(3-bromo-4-cyano-2-hydroxyphenyl)-*N*′-(2-bromophenyl)urea **1f** blocks both IL-8- and GROα-induced chemotaxis[4] of human PMNs with IC_{50} values of 14 and 35 nM, respectively. This result appears to be independent of the ratio of CXCR1 and CXCR2 present on the PMNs, suggesting that neutrophil chemotaxis in humans is predominantly mediated through CXCR2. Rabbits, which also express both CXCR1 and CXCR2 on periferal blood neutrophils, were utilized for in vivo studies. Systemic administration of IL-8 was shown to cause a rapid neutropenia, presumably by effecting the adhesion of neutrophils to vascular endothelial cells. When **1f** was

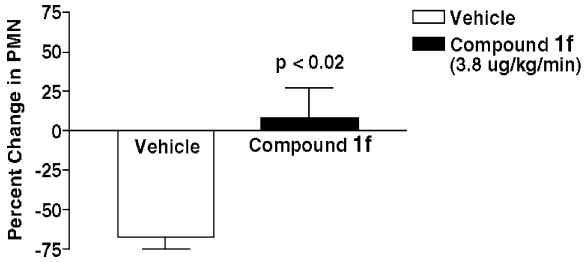


Figure 1. Effects of **1f** on IL-8-induced neutropenia. Rabbits were co-infused with IL-8 (150 ng kg^{-1} min^{-1}) and vehicle or urea **1f** for 30 min via the marginal ear vein. Blood samples were withdrawn at various times over the 30 min infusion via a vascular access port that catheterized the jugular vein. White blood cell counts were determined using a Coulter counter, and differential counts were done using a blood smear stained with DiffQuik. Percent change of PMN count was determined relative to the baseline value. Data shown are the mean ± SEM at the nadir of the neutropenia (5 min).

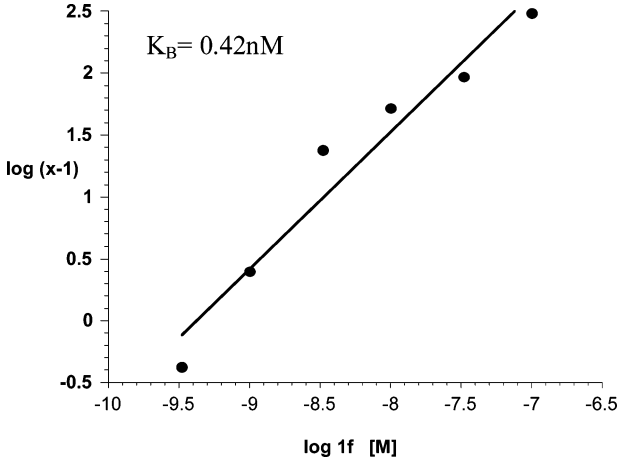


Figure 2. Shild plot for **1f** in GROα-induced calcium mobilization assay. Data were plotted from eight separate GROα-induced calcium mobilization experiments using varying concentrations of GROα with eight fixed concentrations of antagonist.

intravenously co-infused with IL-8, the compound completely blocked the IL-8-induced neutropenia (Figure 1). These phenolic ureas are cleared rapidly in vivo probably because of glucuronidation and/or sulfation of the phenol.[8] Since the compounds are rapidly cleared, their efficacy was evaluated in a topical model of delayed-type hypersensitivity (DTH) where IL-8 has been implicated to mediate the response.[9] In a rabbit model of DTH, the inflammatory response was induced by intradermal injection of tuberculin in animals that had previously been sensitized with complete Freund's adjuvant. Of the four compounds tested in this model (Table 1), ureas **1e** and **1f** both demonstrated significant inhibition (31% and 33%, respectively) at a dose of 500 μg of test compound per injection site.

Conclusion. In summary, our SAR investigation with the phenolic urea series of CXCR2 antagonists has led to the identification of **1f**, a potent and selective agent active both in vitro and in vivo. The activities found with the selective CXCR2 antagonist **1f** indicate that the CXCR2, as opposed to the CXCR1, is more important for neutrophil chemotaxis and recruitment. Ongoing studies with this series of compounds should provide important new information on the roles of CXCR1 and CXCR2 in inflammatory processes.

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Supporting Information Available: Experimental procedures for the preparation of ureas **1a**−**h**. This material is available free of charge via the Internet at http://pubs.acs.org.

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