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# Discovery of potent and orally bioavailable *N*,*N*′-diarylurea antagonists for the CXCR2 chemokine receptor

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**Abstract**—A series of 3-substituted *N*,*N*′-diarylureas was prepared and the structure–activity relationship relative to CXCR2 receptor affinity as well as their pharmacokinetic properties were examined. In vitro microsomal metabolism studies indicated that the lower clearance rates of the 3-sulfonamido-substituted compounds were most likely due to the suppression of glucuronidation.


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 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 containing 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 lymphocytes without apparent pathogenic consequences indicating that these receptors are not required for normal physiology.[3]

Our laboratory has previously disclosed a series of *N*,*N*′-phenylureas, which act as potent and selective CXCR2 antagonists.[4] The early SAR evaluation indicated that substitution at both the 3-position and 4-position on the phenolic-bearing ring was well tolerated. Urea **1** and **2** are representative structures.[5] However, while these compounds displayed high affinity and selectivity for the CXCR2 receptor, poor oral bioavailability and high rates of in vivo clearance were observed. It was thought that this might be due to rapid glucuronidation and/or sulfation of the phenolic function. Therefore, a series of compounds with varying substitution at the adjacent 3-position was prepared to examine its influence on pharmacokinetic properties as well as on receptor affinity.

As a part of this effort, the 3-carboxamide- (**3**), 3-aminomethyl-(**4**), 3-sulfonamide-(**5a**) and 3-*N*,*N*-dimethylsulfonamide-(**5b**) substituted diphenylureas were synthesized.

The synthesis of 3-carboxamide substituted diphenylurea **3** is presented in Scheme 1. Commercially available nitrile **6** was debenzylated to yield the phenol **7**. Nitration with $NaNO_3$ and sulfuric acid resulted in the nitro compound **8**, which after hydrolysis of the nitrile with $H_2SO_4$ yielded the primary amide **9**. Reduction of the nitro group with $SnCl_2$ in EtOH followed by coupling with 2-bromophenyl isocyanate produced the desired urea **3**. As presented in Scheme 2, nitration of 2,6-dichlorobenzyl bromide **10** resulted in the nitro product **11**, which underwent the classical Gabriel procedure to give benzylamine **12**. Following Boc protection of the amine, selective displacement of the chlorine *ortho* to the nitro group was effected by exposure to potassium

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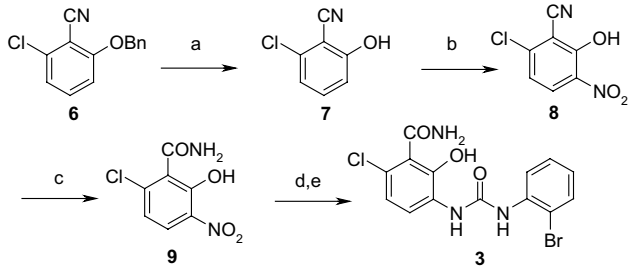
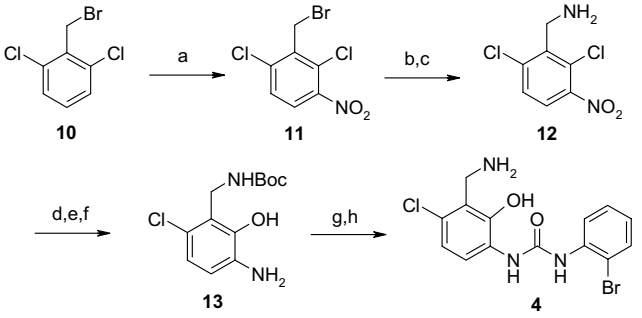
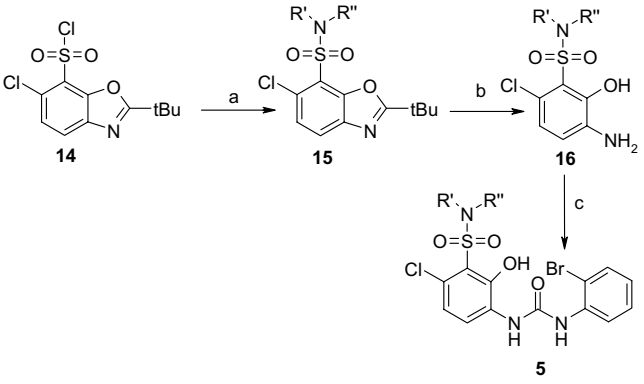


**Scheme 1.** Reagents and conditions: (a) TFA, $CH_2Cl_2$, 66%; (b) $NaNO_3$, $NaNO_2$, $H_2SO_4$, $CH_2Cl_2$, 27%; (c) $H_2SO_4$, 60 °C, 89%; (d) $SnCl_2$, EtOH, 72%; (e) 2-bromophenyl isocyanate, DMF, 73%.



**Scheme 2.** Reagents and conditions: (a) $H_2SO_4$, $HNO_3$, 86%; (b) potassium phthalimide, DMF, rt, 79%; (c) hydrazine hydrate, EtOH, rt, 81%; (d) $(Boc)_2O$, $CH_2Cl_2$, rt, 95%; (e) (i) KOAc, 18-crown-6, DMSO, 100 °C, (ii) NaOH 31%; (f) $H_2$ (g), 10% Pd/C, AcOEt, 92%; (g) 2-bromophenyl isocyanate, DMF, 90%; (h) 4 N HCl in 1,4-dioxane, rt, 34%.

acetate in the presence of 18-crown-6, which after a basic work-up yielded the corresponding phenol. Hydrogenation of the nitro group produced the 2-hydroxy-aniline **13**, which was then coupled with 2-bromophenyl isocyanate. Deprotection of the amine under acidic conditions afforded the desired urea **4**. The preparation of the sulfonamide substituted ureas **5a** and **5b** is outlined in Scheme 3. The synthesis started from the sulfonyl chloride **14**, which was prepared in high yields according to a previously published procedure.[6] This



**Scheme 3.** Reagents and conditions: (a) R′R″NH, $Et_3N$, $CH_2Cl_2$, rt; (R′, R″ = H: 78%; R′, R″ = Me: 74%) (b) 10% aq $H_2SO_4$, reflux ; (R′, R″ = H: 75%; R′, R″ = Me: 63%) (c) 2-bromophenyl isocyanate, DMF; (R′, R″ = H: 62%; R′, R″ = Me: 84%).

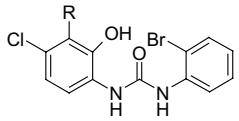
was reacted with either ammonium hydroxide or dimethyl amine to give the sulfonamides **15**, which upon hydrolysis of the oxazole moiety gave the amino-phenols **16**. Treatment with 2-bromophenyl isocyanates resulted in the formation of the final ureas **5a** and **5b**.

The CXCR1 and CXCR2 affinities for the six compounds were determined in SPA-binding assays using [$^{125}$I]-IL-8, and their in vivo pharmacokinetic properties were examined in Sprague–Dawley rats. The results are summarized in Table 1. As seen previously with compounds of this class, a high degree of selectivity for CXCR2 over CXCR1 was observed.[4] Also, electron-withdrawing substituents at the 3-position appeared to be favored, presumably due to the increased acidity of the phenol.[7] However, the fact that the 3-carboxamide **3** is approximately 6-fold more potent than the 3-chloro compound **2** indicates that the acidity of the phenol is not the only determinant of the affinity, since these compounds are predicted to have very similar p$K_a$ values (Table 1). Thus, amide or sulfonamide substituents at the 3-position appear to have additional interactions, which contribute to the binding affinity of these compounds. As the tertiary sulfonamide **5b** displays similar potency to the primary sulfonamide **5a** and the carboxamide **3**, it does not seem to matter whether or not the 3-substituent contains a hydrogen bond donating moiety. Consequently, the observed increase in affinity is most likely due to the amide or sulfonamide acting as hydrogen bond acceptors interacting with one or more residues in the receptor binding site.

The pharmacokinetic properties of the compounds were tested in Sprague–Dawley rats. Despite their common phenolic moiety, these compounds exhibited substantial differences in both clearance and oral bioavailability. Both the unsubstituted urea **1** as well as the 3-chloro substituted compound **2** were rapidly cleared and showed little oral bioavailability. The 3-carboxamide-substituted urea **3** was prepared to examine the influence of a polar moiety adjacent to the 2-phenol. However, this compound only showed a slight decrease in clearance when compared to the unsubstituted analog **1**. Similarly, placement of a charged group at the 3-position, in the form of a aminomethyl moiety (**4**), did little to reduce clearance, but did improve the oral bioavailability. In contrast, introduction of sulfonamide substituents at the 3-position substantially decrease clearance and increased oral bioavailability. Thus, urea **5a** was cleared approximately 13 times slower than that of unsubstituted compound **1**, and 6 times slower than the 3-carbox-amide **3**.

Further in vitro metabolism studies using rat and human hepatic microsomes were carried out in the presence and absence of uridine diphosphate glucuronic acid (UDPGA), to discern the role of glucuronidation in metabolism. As outlined in Table 2, clearance of the carboxamide **3** was low, but was significantly enhanced in the presence of UDPGA, demonstrating the important role of glucuronidation in the clearance of this amide. However, the intrinsic clearance of sulfonamide **5a** was only minimally enhanced in the presence of

**Table 1.** CXCR1 and CXCR2 binding affinities, pharmacokinetic properties for ureas **1**–**5b**




| Compound | R | CXCR2[a] ($IC_{50}$, nM) | CXCR1[a] ($IC_{50}$, nM) | CL[b] (mL/min/kg) | Oral $F$ (%) | $c\mathrm{Log}\,D$[c] | p$K_a$ (calcd)[d] |
|---|---|---|---|---|---|---|---|
| **1** | H | 906 | <30,000 | 323 | 6.6 | 3.85 | 8.24 |
| **2** | Cl | 63 | 13,360 | 73.6 | 13 | 4.02 | 7.24 |
| **3** | $CONH_2$ | 10 | 6037 | 105 | 3.6 | 2.43 | 7.11 |
| **4** | $CH_2NH_2{\cdot}HCl$ | 114 | 30,000 | 104 | 67 | 0.33 | 6.85 |
| **5a** | $SO_2NH_2$ | 7 | 1366 | 16.1 | 86 | 2.05 | 6.12 |
| **5b** | $SO_2NMe_2$ | 12 | 4317 | 23.4 | 100 | 3.23 | 6.19 |

Abbreviations: CL: systemic plasma clearance; F: percent bioavailability.

[a] Binding assays were performed on membranes of 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.[8] All assays were performed in 96-well microtiter plates using radio-labeled [$^{125}$I]-IL-8 (human recombinant; concentration: 0.23 nM). The binding results are expressed as a mean of three individual experiments.

[b] Pharmacokinetic experiments were conducted using an iv×po crossover design in male Sprague–Dawley rats ($n=3$ per study). Compounds were administered in an aqueous solution containing 1% DMSO and up to 20% hydroxypropyl-beta-cyclodextrin at dosages of 4 and 8 μmol/kg for iv and po doses. Blood samples were obtained from a lateral tail vein and plasma analyzed for drug content using LC/MS/MS methodologies, with a lower limit of quantification of >10 ng/mL for each analyte.

[c] $c\mathrm{Log}\,D$ values were calculated using MANTIS 2.1.0 software based on the Daylight/PCModels 4.81 package supplied by Daylight Chemical Information Systems, Los Altos.

[d] p$K_a$ values were calculated using ACD/p$K_a$ software supplied by Advanced Chemical Development, Toronto.

**Table 2.** In vitro intrinsic clearance of ureas **3** and **5a** in rat and human liver microsomes

| Compound | $Cl_{int}$ (mL/min/g liver)[a] | | $Cl_{int}$ (mL/min/g liver)[a] | |
|---|---|---|---|---|
| | Rat | | Human | |
| | (−) UDPGA | (+) UDPGA | (−) UDPGA | (+) UDPGA |
| **3** | 1.5 | 5.3 | 0.74 | 15 |
| **5a** | Stable | 1.6 | Stable | 2.7 |

[a] In vitro intrinsic clearance experiments were conducted in commercially purchased rat or human liver microsomes at 0.5 mg microsomal protein/mL incubation and final compound concentrations of 0.5–1 μM. Disappearance of substrate was monitored over a 30-min incubation by LC/MS/MS and the intrinsic clearance ($CL_{int}$) calculated from the slope of the substrate disappearance versus time curve.

UDPGA, and was approximately 3- to 6-fold lower than that of amide **3**. These data suggest that exchanging the amide substituent for a sulfonamide increases resistance to glucuronidation, which likely contributes to the reduced clearance and improved oral bioavailability of these compounds. A survey of the literature on UDP-glucuronyltransferase substrate specificity[9] points to two factors, which may be responsible for this result: First, UDP-glucuronyltransferases appear to prefer planar phenols[10] and thus the more three-dimensionally bulky sulfonamide group may adversely affect the rate of glucuronidation. Secondly, the nucleophilicity of the phenol has also been shown to influence the rate of glucuronide transfer.[11] Therefore, the greater electron-withdrawing capacity of the sulfonamide relative to the carboxamide likely adds to the suppression of glucuronidation. Finally, substrate lipophilicity is often mentioned as a key determinant of glucuronidation rate, however, in this case it seems to be less significant as judged by the calculated log $D$s (Table 1).

In summary, placement of a 3-sulfonamido substituent on the previously described diaryl urea scaffold resulted in the discovery of a new class of potent and selective CXCR2 receptor antagonists with greatly improved pharmacokinetic properties. In vitro studies performed with hepatic microsomes indicate that the enhanced pharmacokinetic properties of these compounds may be a result of decreased susceptibility to phenolic glucuronidation. Compounds of this type should prove highly useful for the investigation of the pharmacology related to the CXCR2 receptor in vivo.

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