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Nicotinamide *N*-Oxides as CXCR2 Antagonists

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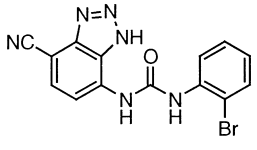
Abstract—A series of nicotinamide *N*-oxides was synthesized and shown to be novel, potent, and selective antagonists of the CXCR2 receptor. Furthermore, these compounds showed significant functional activity against GRO-α-driven human neutrophil chemotaxis. Compounds of this class may be useful for the treatment of inflammatory, auto-immune, and allergic disorders.

Introduction

Chemokines selectively recruit and activate a variety of cells during inflammation. Interleukin-8 (IL-8) and other Glu-Leu-Arg (ELR)-containing CXC chemokines, including epithelial cell-derived neutrophil-activating peptide-78 (ENA-78), and growth-related oncogene (GRO)-α, GRO-β, and GRO-γ, attract and activate neutrophils. In response to traumatic injury or infection, human neutrophils are directed to the site of injury or infection by CXC chemokines that signal via two distinct seven-transmembrane G-protein coupled receptors (GPCRs), CXCR1 and CXCR2. Both CXCR1 and CXCR2 bind IL-8 with high affinity; however, only CXCR2 binds the other neutrophil-activating and ELR motif-bearing chemokines with high affinity. Although the exact role of IL-8 and GRO-α in human disease is yet to be determined, these and other pro-inflammatory chemokines are observed in association with chronic and acute inflammatory conditions and correlate with neutrophil infiltration in a wide range of diseases including psoriasis, rheumatoid arthritis, and adult respiratory distress syndrome.[1]

Small-molecule CXCR2 antagonists are potentially valuable novel therapeutics, although relatively few have been reported in the literature. Most noteworthy is compound **1** that has demonstrated potent inhibition of neutrophil chemotaxis ($IC_{50} = 10$ nM) and was efficacious in vivo in an LPS-induced rabbit model of neutrophilia.[2] Compound **2** was recently claimed as a selective antagonist of IL-8 binding ($IC_{50} = 110$ nM) and potent inhibitor of neutrophil chemotaxis ($IC_{50} = 170$ nM).[3]

In this paper, we report the preliminary results of a project to discover selective antagonists of the CXCR2 receptor. An effort to identify inhibitors of chemokine-driven neutrophil responses found compound **4a**, a nicotinamide *N*-oxide with promising activity. A series of studies were then conducted to define the SAR of these compounds as potential new antiinflammatory therapeutics.



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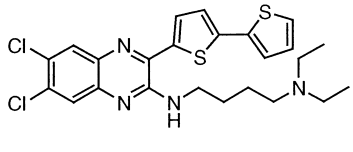

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Chemistry

6-Chloronicotinamide *N*-oxides were prepared according to the route shown in Scheme 1. Commercially available 6-chloronicotinic acid was converted to the corresponding *N*-oxide **3** using trifluoroperacetic acid.[4] Acid **3** was subsequently reacted with a variety of amines or anilines using EEDQ as the coupling reagent of choice to provide compounds **4a**–**h**.

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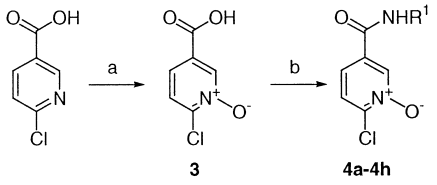
Scheme 2 outlines two synthetic routes toward 6-sulfonyl-1-oxy-nicotinamides. 6-Chloronicotinoyl chloride was reacted with 4-fluoroaniline to provide nicotinamide **5** in high yield. Route A was used to readily oxidize the pyridine core using trifluoroperacetic acid[4] at elevated temperatures to provide **4a**. Efforts to effect the oxidation with other oxidizing reagents such as *m*-CPBA,[5] *m*-CPBA in the presence of HF,[6] magnesium monoperoxyphthalate hexahydrate,[7] or urea hydrogen peroxide complex and phthalic anhydride in methanol failed to provide appreciable quantities of desired product. Displacement of the 6-chloro substituent with sodium methanesulfinate provided analogue **7a**. Because of the limited number of available sulfinates, a second synthetic strategy was developed (route B). Compound **5** could be reacted with a variety of alkyl or aryl potassium thiolates to provide the corresponding 6-thionicotinamides **6**. Aqueous trifluoroperacetic acid was then used as the preferred oxidant to provide compounds **7b–g**. This single-step, novel oxidation of both the pyridine core to the corresponding *N*-oxide and the thioether to the corresponding sulfone was readily monitored by HPLC–MS. The oxidation reactions were typically complete within 12 h at which time the products precipitated from solution. The 6-sulfonyl-1-oxy-nicotinamides were then readily purified via trituration to afford the products in yields >50%.

Results and Discussion

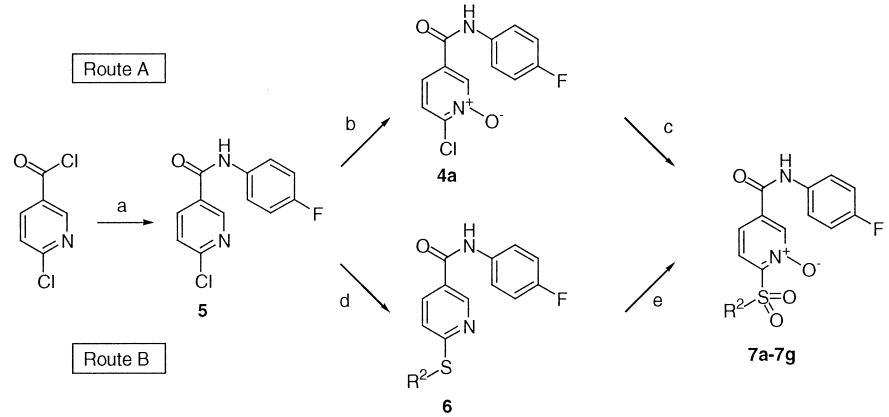
As part of a lead optimization strategy, nicotinamide *N*-oxides were screened for both their ability to antagonize the binding of IL-8 to CXCR2 and for their ability to block GRO-α-driven human neutrophil chemotaxis.[8]



Scheme 1. Reactions and conditions: (a) TFA, 30% H_2O_2 in H_2O, 0–45 °C, 64%; (b) R^1NH_2, EEDQ, $CHCl_3$, rt.

Ligand binding was evaluated using an SPA assay with recombinant human [^{125}I]-IL-8 and membranes prepared from Sf9 cells expressing human CXCR2 and Gαi3β1γ2 proteins.[9] [^{125}I]-IL-8 was chosen as the ligand in the binding assay because it gave more reproducible results than did [^{125}I]-GRO-α.

SAR studies initially focused on amide modifications in the 3-position of the pyridine (Table 1). It was found that a number of substituted anilides blocked GRO-α-driven neutrophil chemotaxis with activities in the low micromolar range. Efforts to replace the anilide functionality with other amides, however, led to compounds with significantly decreased functional activity as illustrated by compounds **4g** and **4h.** In contrast to the SAR flexibility observed for a variety of anilides in the chemotaxis assay, no replacement of the 4-fluoroanilide was found that gave acceptable activity in the IL-8 ligand binding assay. Based on our SAR survey, the 4-fluoroanilide moiety of compound **4a** was shown to be a critical recognition element necessary for blocking IL-8/CXCR2 interaction. The disparity between ligand binding affinity and functional activity is not without precedent in the chemokine field. It was shown by Jarnagin et al. that mutants of the CC chemokine, monocyte chemotactic protein, could function as high-affinity binders to CCR2, yet were functionally inactive to stimulate chemotaxis.[10] It was also recently demonstrated that high-affinity binding of IL-8 and GRO-α to CXCR2 involves interaction with specific and different amino acid residues of CXCR2. It was proposed that the CXCR2 amino acid residues required for cell activation are not necessarily the same residues required for ligand binding.[11] Related work by Ahuja et al. demonstrated that there are divergent regions of ligand contact on CXCR2 for binding as well as cell activation and concluded that distinct antagonists could be found to independently block either high affinity binding or receptor activation or both.[12] Based on the possibility of multiple binding domains for a small molecule with CXCR2, there are several possible explanations for the disparity between the functional and ligand binding activities for the compounds in this series. Compounds **4b–e** may interact at specific regions of CXCR2 that block GRO-α but not IL-8 binding. Alternatively,



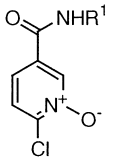
Scheme 2. Reactions and conditions: (a) 4-$FC_6H_4NH_2$, *i*-Pr_2NEt, CH_2Cl_2, 0 °C to rt, 92%; (b) 30% H_2O_2 in TFA, 0–45 °C, 25%; (c) $NaSO_2Me$, DMF, 56%; (d) R^2SH, *t*-BuOK, THF, 60–80%; (e) 30% H_2O_2 in TFA, 0–45 °C, 50–70%.

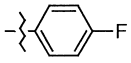
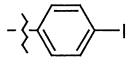
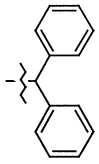
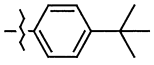
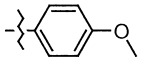
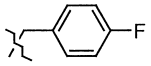
compounds **4b–e** may inhibit neutrophil chemotaxis through interactions with amino acid residues on CXCR2 that are required for receptor activation but not required for agonist binding.

As a complement to SAR exploration at the 3-position of the nicotinamide *N*-oxides, it was of interest to look at SARs around the 6-substituent of the *N*-oxide core. It was found that 6-sulfonylnicotinamide *N*-oxides were particularly interesting (Table 2). In general, a diverse set of 6-alkyl and -alkylaryl substituted nicotinamide *N*-oxide sulfones provided potent analogues, especially in the ligand binding assay. As was observed with the 6-chloronicotinamide *N*-oxides however, there were examples of compounds that had disparate activity between their binding and functional activities. In particular, the aryl sulfones **7e** and **7f** represented the most potent ligand binding antagonists in the series with IC_{50}'s of 90 and 32 nM, respectively, yet they were functionally inactive. Again, these compounds may interact at sites on CXCR2 that specifically disrupt IL-8 binding or bind at a subsite of CXCR2 not required for GRO-α-stimulated receptor activation.

As part of an ongoing assessment of these compounds, the selectivity of representative 6-chloro- and 6-sulfonylnicotinamide *N*-oxides was investigated. Ligand binding antagonist selectivity was assessed using a panel of phylogenetically diverse GPCRs (Table 3). In all cases, the compounds were most effective in the CXCR2 assay. In general, little or no activity was seen at the non-chemokine receptors. Compounds **4a** and **7a** did inhibit binding of IL-8 to CXCR1, but to a lesser extent than at CXCR2. The observation that **7b**, **7d**, and **7g** were highly selective for CXCR2 versus CXCR1 suggests that the compounds were in fact interacting with CXCR2 rather than binding to the chemokine IL-8. Functional selectivity was assessed in a neutrophil chemotaxis assay using *N*-formyl-methionyl-leucyl-phenylalanine (FMLP). This chemoattractant polypeptide activates neutrophils via specific *N*-formyl peptide receptors. In all cases, the compounds were more potent as GRO-α antagonists than as FMLP antagonists. Again, compound **4a** was unique in that it displayed the highest overall level of ligand binding and functional selectivity.

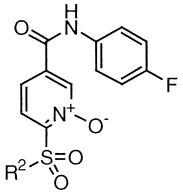
Table 1. Representative activities of 6-chloronicotinamide *N*-oxides

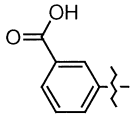
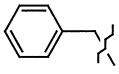


Compound	R^1	GRO-α chemotaxis[a] (IC_{50}, μM)	IL-8 SPA binding[a] (IC_{50}, μM)
4a		1.1	1.0
4b		0.5	>20
4c		0.83	>20
4d		1.3	>20
4e		4.2	>20
4f		>20	>20
4g		>20	>20
4h		>20	>20

[a]Values were calculated from the geometric mean of at least two experiments.

Table 2. Representative activities of 6-sulfonylnicotinamide *N*-oxides



Compound	R^2	GRO-α chemotaxis[a] (IC_{50}, μM)	IL-8 SPA binding[a] (IC_{50}, μM)
7a		2.0	0.13
7b		3.3	0.13
7c		2.0	0.40
7d		1.9	0.46
7e		>20	0.090
7f		>20	0.032
7g		1.0	0.28

[a]Values were calculated from the geometric mean of at least two experiments. An IC_{50} value of >20 indicates less than 50% activity at 20 μM.

Table 3. Percent inhibition of binding[a,b] at 20 μM and FMLP neutrophil chemotaxis inhibition[a] of representative nicotinamide *N*-oxides

Compound	CXCR1[c]	CXCR2[d]	Calcitonin[e]	Cholecystokinin CCK_B[f]	Endothelin ET_B[g]	Neuropeptide Y_1[h]	Somatostatin[i]	FMLP (chemotaxis[j] IC_{50}, μM)
4a	68	85	−1	11	14	15	-2	>40
7a	73	97	−20	40	8	58	60	9.6
7b	8	97	3	14	15	41	−15	13.3
7d	8	60	4	1	−2	−1	−19	NT[k]
7g	9	77	−23	29	8	32	38	13

[a]Values were calculated from the geometric mean of at least two experiments.
[b]Binding assays were conducted at Panlabs (Bothell, WA).
[c]Displacement of [^{125}I]-IL-8 from CHO cell membranes expressing human CXCR1 receptors.
[d]Displacement of [^{125}I]-IL-8 from CHO cell membranes expressing human CXCR2 receptors.
[e]Displacement of [^{125}I]-calcitonin (salmon) from T-47D (human breast cancer) cell membranes expressing human calcitonin receptors.
[f]Displacement of [^{3}H]-CCK-8 from NIH-3T3 (mouse embryo cell membranes) expressing human cholecystokinin CCK_B receptors.
[g]Displacement of [^{125}I]-endothelin-1 from CHO cell membranes expressing human endothelin ET_B receptor.
[h]Displacement of [^{125}I]-peptide YY (PYY) from SK-N-MC (human neuroblastoma) cell membranes expressing human neuropeptide Y_1 (NPY_1) receptors.
[i]Displacement of [^{125}I]-tyr^1 somatostatin from AtT-20 (mouse pituitary) cell membranes expressing somatostatin receptors.
[j]See ref 8.
[k]Not tested.

Since compound **4a** was unique in that it showed appreciable selectivity over other GPCRs as well as activity as both a ligand binding and functional antagonist, additional studies were undertaken. It was shown that **4a** was able to moderate IL-8 driven human neutrophil chemotaxis (IC_{50} = 1.3–2.3 μM), was well tolerated in mice at 100 mg/kg po, and was relatively stable to rat liver microsomes (data not shown).

In conclusion, the synthesis and evaluation of a novel series of potent and selective CXCR2 antagonists has been described. The molecules have shown potency in an IL-8 ligand binding assay and show the ability to block neutrophil chemotaxis using GRO-α as the agonist. Compound **4a** was selected for more advanced investigation based on its in vitro functional, ligand binding, and pharmacological profile. The nicotinamide *N*-oxides continue to provide valuable tools in the investigation of the role of CXCR2 on neutrophils.

Acknowledgements

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