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Carboxylic acid bioisosteres acylsulfonamides, acylsulfamides, and sulfonylureas as novel antagonists of the CXCR2 receptor

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Abstract—A series of novel acylsulfonamide, acylsulfamide, and sulfonylurea bioisosteres of carboxylic acids were prepared as CXCR2 antagonists. Structure–activity relationships are reported for these series. One potent orally bioavailable inhibitor had excellent PK properties and was active in a lung injury model in hyperoxia-exposed newborn rats.

Recruitment of neutrophils and monocytes is a normal physiological response to infection and tissue damage. However, excessive numbers of these cells can produce additional tissue damage by releasing proteases, oxygen radicals, and other mediators. Proteases in particular can stimulate mucus secretion,[1] and other airway events associated with COPD,[2] acute respiratory distress syndrome,[3] asthma,[2,4] chronic bronchitis,[5] pulmonary fibrosis,[6] and cystic fibrosis.[7] CXC chemokines that contain the sequence Glu-Leu-Arg (ELR) before the first N-terminal cysteine residue mediate, in part, the recruitment of neutrophils and a subset of monocytes. ELR+ chemokines act through CXC chemokine receptors CXCR1 and CXCR2. CXCR2 is selectively stimulated by chemokines GRO-α, -β, and -γ, NAP-2, ENA-78, while IL-8 and GCP-2 stimulated both CXCR2 and CXCR1.[8,9] There is some contention whether human neutrophil migration is mediated by IL-8 activation of one or both CXC receptors, but studies with a selective CXCR2 antagonist (SB-225002) indicate that neutrophil chemotaxis may primarily be an effect of CXCR2 induction.[10]

Inhibitors of CXCR2 have been disclosed by several groups of workers recently.[11] In addition, a noncompetitive allosteric inhibitor of CXCR1 and CXCR2, repertaxin, is in Phase II clinical trials for the prevention of reperfusion injury.[12] Indolylbuteric acid **1** has been reported as a sub-micromolar inhibitor of CXCR2 (Fig. 1).[13] With the hope of identifying an orally bioavailable series of CXCR2 inhibitors, we evaluated series of acylsulfonamides **2** (R^1 is C-linked) and acylsulfamides **2** (R^1 is N-linked), and a series of sulfonylureas **3**, as carboxylic acid bioisosteres of **1**.[14] Other acid bioisosteres, such as tetrazoles, were found to greatly reduce potency and were not pursued further.

The general synthetic schemes to the acylsulfonamides and acylsulfamides described are shown below (Fig. 2).

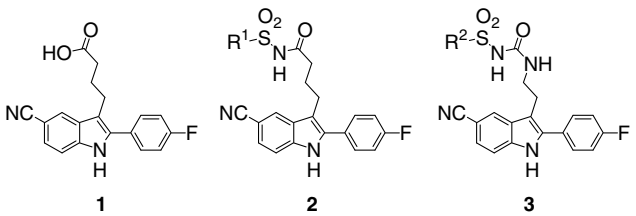

Figure 1. Acylsulfonamide, acylsulfamide, and sulfonylurea-based CXCR2 inhibitors.

Keywords: Carboxylic acid bioisosteres; Acylsulfonamides; Acylsulfamides; Sulfonylureas; CXCR2; Chemokine; IL-8; CXCR1; Indole; Neutrophils; COPD; Asthma.
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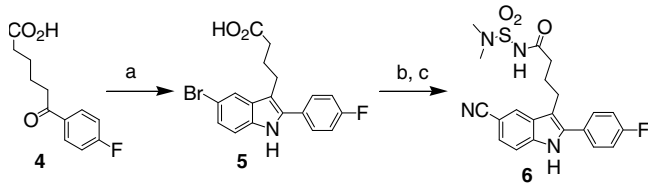


Figure 2. General acylsulfonamide and acylsulfamide synthesis. Reagents and conditions: (a) 4-bromophenylhydrazine-HCl, $ZnCl_2$, HOAc, 70 °C; (b) CuCN, NMP, 200 °C, μwave; (c) $Me_2NSO_2NH_2$, EDC, DMAP, THF, DCM.

Starting with aryl ketone **4**, treatment with 4-bromophenylhydrazine hydrochloride and $ZnCl_2$ in acetic acid at 70 °C afforded indole **5**.[13a] Conversion of the bromo to cyano with CuCN followed by EDC coupling of the acid with dimethylsulfamide gave **6**.[15]

A second general route was used to regioselectively synthesize the 5,6-disubstituted indole derivatives (Fig. 3). Reduction of 2-methoxy-4-nitrobenzonitrile **7** with sodium hydrosulfite followed by regioselective iodination with *N*-iodosuccinimide furnished iodoaniline **8**. Palladium coupling of **8** with TMS-acetylene **9** gave the 2-TMS-indole **10**,[16] which was subsequently converted to the 2-iodoindole with iodine monochloride and then to the 2-(4-fluorophenyl) derivative **11** by Suzuki coupling. Acylsulfonamide and acylsulfamide derivatives of the 5,6-disubstituted indoles were synthesized using chemistry described in Figure 2.

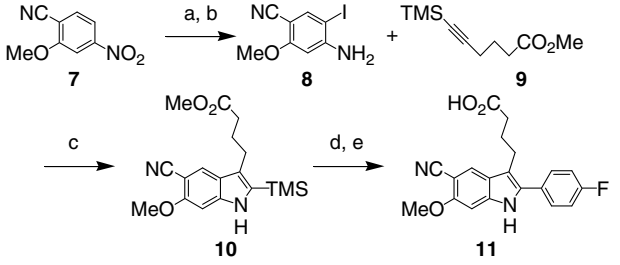


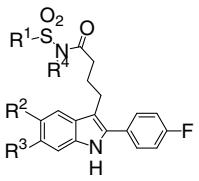
Figure 3. General acylsulfonamide and acylsulfamide synthesis. Reagents and conditions: (a) $Na_2S_2O_4$, THF, H_2O; (b) NIS, HOAc; (c) $PdCl_2$-DPPF-DCM, LiCl, Na_2CO_3, DMF, 100 °C; (d) ICl, DCM, 0 °C to rt; (e) 4-fluorophenylboronic acid, $Pd(OAc)_2$, tri-*o*-tolylphosphine, Na_2CO_3, DME/H_2O/EtOH, 80 °C.

Shown last is the synthesis of sulfonylurea derivative **16** (Fig. 4). The chloro of **12** was displaced by phthalimide to give **13**, which can then be converted to indole **14** using Fisher indole chemistry described in Figure 2. The 5-bromoindole **14** was converted to the 5-cyano, and the phthalimide group removed with hydrazine to give **15**. Treatment with carbonyldiimidazole gave the isocyanate, which was converted onto the sulfonylurea **16** by reaction with benzenesulfonamide and potassium carbonate.

Early on, we found that the SAR of the acid mimetics and the acids was quite similar in the CXCR2 binding assay.[17] At the 5-position of the indole, only CN and Br were found to be active. At the 2-position of the indole, only halogen-substituted aryl was active. These positions were left constant in the subsequent SAR investigation.

We first explored the SAR around the acylsulfonamide bioisostere (Table 1). The first compound made, the methyl-substituted acylsulfonamide (**17**), was 72 nM. First, replacement of the acidic N–H with N–Me (**18**) resulted in a complete loss of potency confirming the necessity of an acidic proton at R^4 to activity. Replace-

Table 1. SAR of acylsulfonamide derivatives



Compound	R^1	R^2	R^3	R^4	CXCR2 IC_{50} (μM)
17	Me	CN	H	H	0.072
18	Me	CN	H	Me	>2.0
19	Me	Br	H	H	0.170
20	Et	CN	H	H	0.064
21	*n*-Pr	CN	H	H	1.30
22	Bn	CN	H	H	1.40
23	*i*-Pr	CN	H	H	0.22
24	Ph	CN	H	H	0.260
25	CF_3	CN	H	H	0.087
26	Me	CN	OMe	H	0.16
27	Me	CN	Me	H	0.019

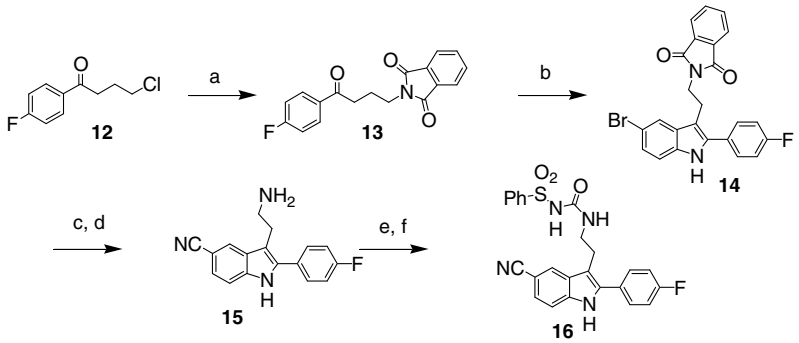


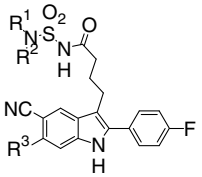
Figure 4. General sulfonylurea synthesis. Reagents and conditions: (a) potassium phthalimide, DMF, 100 °C; (b) 4-bromophenylhydrazine-HCl, $ZnCl_2$, HOAc, 70 °C; (c) hydrazine, EtOH, 80 °C; (d) CuCN, NMP, 200 °C, μwave; (e) CDI, CH_3CN; (f) $PhSO_2NH_2$, K_2CO_3, acetone, 60 °C.

ment of the 5-cyano with 5-bromo (**19**) at R^2 resulted in an approximate twofold loss in potency. Steric factors on the acylsulfonamide R^1 substituent appear to be quite important; ethyl (**20**) and methyl are preferred, but *n*-propyl (**21**) and benzyl (**22**) gave almost a 20-fold loss in potency. Isopropyl (**23**) and phenyl (**24**) substitution demonstrate that branching at the α-carbon is tolerated, although not preferred. Replacement of the methyl with CF_3 (**25**) gave an almost equipotent compound indicating that electron-withdrawing substituents are tolerated. Finally, a brief investigation into 5, 6-disubstituted indoles (R^3 substitution) revealed that the 6-methoxy (**26**) gave a twofold drop in potency while the 6-methyl (**27**) gave almost a fourfold improvement in potency, 19 nM against CXCR2.

The activity of the acylsulfonamide series prompted the investigation of the acylsulfamide series (Table 2). The first compound made (**6**) had an IC_{50} of 50 nM, and led to an exploration of the SAR of this novel series. Removal of one methyl (**28**) gave more than a twofold loss in potency, however, removal of the second methyl (**29**) restored much of that loss in potency. As in the acylsulfonamide series, steric factors are important; replacement of the dimethyl with diethyl (**30**) or *n*-butyl (**31**) gave a dramatic decrease in potency, as did phenyl (**32**). However, the methoxyethyl (**33**) gave a much smaller loss in potency compared to the similarly sized *n*-butyl implying an electronic factor may be important. The morpholine substitution (**34**) appears to confirm this electronic benefit when compared to the diethyl (**30**). As in the acylsulfonamide series, substitution at the 6-position of the indole with methoxy (**35**) gave an equipotent compound while methyl (**36**) gave the most potent compound in the series, 24 nM.

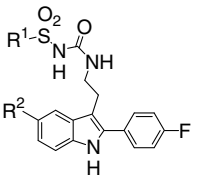
Next, we investigated the sulfonylurea series (Table 3). As described below, the SAR of this series differed considerably from the previous two series. The first compound made, where R^1 is methyl and R^2 is bromo (**37**), had an IC_{50} of 0.25 μM against CXCR2. Unlike the acylsulfonamide series, replacing the R^2 bromo with cyano (**38**) resulted in a two- to threefold loss in potency. Substitution of the methyl at R^1 with phenyl on both the bromo (**39**) and cyano indoles (**16**) gave improved potency with IC_{50}s of 0.12 and 0.14 μM, respectively. The sulfonylureas most likely have a different conformation than either the acylsulfonamides or acylsulfamides accounting for the preference for phenyl at R^1. *Ortho*-(**40**) and *para*-(**41**) halogen substitution on the phenyl are both disfavored.

Table 2. SAR of acylsulfamide derivatives



Compound	R^1	R^2	R^3	CXCR2 IC_{50} (μM)
6	Me	Me	H	0.050
28	Me	H	H	0.12
29	H	H	H	0.066
30	Et	Et	H	1.10
31	*n*-Butyl	H	H	1.10
32	Ph	H	H	0.88
33	$-CH_2CH_2OMe$	H	H	0.26
34	Morpholine		H	0.15
35	Me	Me	OMe	0.057
36	Me	Me	Me	0.024

Table 3. SAR of N-linked sulfonylurea derivatives



Compound	R^1	R^2	CXCR2 IC_{50} (μM)
37	Me	Br	0.25
38	Me	CN	0.64
39	Ph	Br	0.12
16	Ph	CN	0.14
40	*o*-Cl-phenyl	CN	0.40
41	*p*-F-phenyl	CN	0.52

Compounds from each series were selected for further evaluation in a CXCR2 calcium flux FLIPR assay,[18] and a rabbit neutrophil chemotaxis assay (Table 4).[19] While there does not appear to be a strong correlation between the absolute numbers in the CXCR2 binding assay and the secondary cellular assays, there is a general trend indicating that the more potent compounds in the binding assay were among the more potent compounds in both the FLIPR assay and the neutrophil chemotaxis assay.

Due to its low IC_{50} in all three assays, compound **6** was chosen for further testing. In a screen of cytochrome P450s, **6** had an IC_{50} of 1.32 μM versus Cyp2C9, and >5 μM for isoforms 1A2, 3A4, 2C19, and 2D6. **6** also had excellent human and rat liver microsomal stability, 100% remaining at 10 min in rat and 76.8% remaining in human. Plasma-protein binding was very high, >99% for human and rat. Pharmacokinetic studies of **6** in rats revealed an iv $t_{1/2}$ of 2.7 h, very low clearance of 3.8 mL/min/kg, and excellent oral bioavailability of 107%.

Table 4. CXCR2 FLIPR and rabbit neutrophil chemotaxis assays

Compound	CXCR2 IC_{50} (μM)	CXCR2 FLIPR IC_{50} (μM)	Rabbit neutrophil chemotaxis IC_{50} (μM)
6	0.050	0.005	0.7
28	0.12	0.014	1.3
17	0.072	0.032	2.1
25	0.087	0.070	3.0
39	0.12	0.004	1.7
37	0.25	0.090	5.8

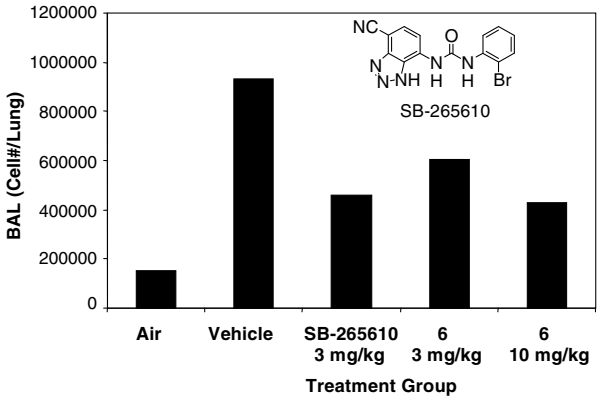


Figure 5. Effects of CXCR2 Inhibitor **6** on hyperoxia-induced neutrophil accumulation in newborn rats.

Following the excellent PK results, **6** was chosen for a lung injury model of hyperoxia-induced neutrophil accumulation in newborn rat lungs and compared to a known positive control, CXCR2 inhibitor SB-265610 (Fig. 5).[20] Compound **6** at 10 mg/kg given intraperitoneally showed equivalent reduction, approximately 50%, of hyperoxia-induced neutrophil accumulation in bronchoaveolar lavage (BAL) to SB-265610 at 3 mg/kg.

In conclusion, we identified multiple series of potent inhibitors of CXCR2. One example, compound **6**, has been shown to have excellent oral bioavailability and has demonstrated good activity in vivo in a rat model of lung injury.

Acknowledgments

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17. DELFIA binding assays measured binding of Europium-labeled human IL-8 to cloned human CXCR2 receptor sf9 membrane lysates coexpressed with $G\alpha_{i3}\beta_1\gamma_2$ proteins (Perkin-Elmer). In a reaction volume of 100 μL in 96-well 0.45 μm HV filtration plates (Millipore) test compound in assay buffer (50 mM Tris, pH 7.5, 5 mM $MgCl_2$, 25 μM EDTA, 0.2% BSA, 50 μg/mL saponin, 2 mM $CaCl_2$) was mixed, in triplicate, with 2 nM Eu-IL-8. Reaction was initiated by addition of 40 μg/mL suspended lysates, and incubated for 90 min at room temperature. Plates were washed 4 times with DELFIA L*R wash buffer (Perkin-Elmer), (200 μL/well). DELFIA Enhancement Solution (Perkin-Elmer, 200 μL/well) was added to the plate, the plate was shaken for 15 min at room temperature, and time-resolved fluorescence was recorded at 340 ex/612 em with a 400 μs delay. Binding response was a measure of maximal fluorescent signal from background (200 nM unlabeled human IL-8, Biosource), and IC_{50}s were calculated using GraphPad Prism® software and a four-parameter logistics equation.
18. IL-8 induced calcium flux (FLIPR, Molecular Devices) was performed with Chem-1 cells (Chemicon) that contain a cloned human CXCR2 coupled to Gi/o protein. Confluent cells (100 μL of 5×10^5 cells/mL) were allowed to adhere to 96-well black, clear-bottom plates overnight in DMEM without G-418. Growth media was replaced with 100 μL half-strength Calcium 3 fluorescent dye (Molecular Devices) in assay buffer (Hank's balanced salt solution with Ca^{+2} and Mg^{+2}, 20 mM Hepes, pH 7.4, 2.5 mM probenecid), 50 μL test compound in assay buffer, in triplicate, and incubated at 37 °C for 30 min, followed by an additional 30 min at room temperature. The reaction was initiated by addition of 50 μL human IL-8 (R&D Systems, 4 nM in assay buffer with 0.25% BSA), and fluorescence (485 ex/525 em) was monitored every 2 s for

1 min. Agonist response was a measure of maximal fluorescent signal from background, and IC_{50}s were determined as for Ref. 17.

19. Rabbit neutrophil chemotaxis was performed using a variation of the method described by Frevert.[21] Neutrophils were isolated from whole rabbit blood by centrifugation on lymphocyte separation media, Accupaque (Accurate Chemical). RPMI media containing 0.1% BSA with or without 38 nM GRO-α (R&D Systems) was preincubated in the bottom chamber of a 3 μm 96-well ChemTx plate (Neuroprobe) for 1 h at 37 °C. Twenty-five microliters of RPMI media containing 0.1% BSA and rabbit neutrophils (3×10^6 cells/ml) were mixed with an equal volume of media containing test compound and applied to quadruplicate wells on the top of the membrane, and incubated at 37 °C for 1 hour. Cells migrating into the bottom chamber were measured against background (no GRO-α) using Cell Titer Glo (Promega), and IC_{50}s were determined as for Ref. 17.
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