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Synthesis and structure–activity relationships of 3,4-diaminocyclobut-3-ene-1,2-dione CXCR2 antagonists

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Abstract—A novel series of 3,4-diaminocyclobut-3-ene-1,2-diones was prepared and found to show potent inhibitory activity of CXCR2 binding and IL-8-mediated chemotaxis of a CXCR2-expressing cell line. Microsome stability and Caco2 studies were subsequently used to show that compounds of this chemotype are predicted to have good oral bioavailability and are thus suitable for pharmaceutical development.

Inflammatory disease is mediated principally via the migration of leukocytes into the affected tissues.[1] This influx is sustained by chemotactic cytokines, otherwise known as chemokines.[2] Neutrophils are a subset of leukocytes implicated in the etiology of conditions such as chronic obstructive pulmonary disease (COPD),[3,4] rheumatoid arthritis (RA),[3,5] inflammatory bowel disease,[3,6] sepsis,[3,7] and psoriasis.[3,8] CXCL8 (formerly interleukin-8 or IL-8) is a chemoattractant for neutrophils that is readily detected at sites of inflammation.[9] CXCR1 and CXCR2 are the only known neutrophil receptors that contain high-affinity binding sites for CXCL8.[10] Specific roles for each receptor remain elusive, but a correlation between receptor activation and neutrophil-trafficking has been firmly established through animal models of human disease using either CXCR2 knockout mice,[11] monoclonal antibodies,[12] and CXCR2 antagonists.[13] Such promising results make the development of CXCL8 receptor antagonists an intriguing opportunity for pharmaceutical development.

The series of ureas (Fig. 1) developed by researchers at GlaxoSmithKline (GSK)[14] has recently garnered much attention, as an analog related to this series is now in human trials for the treatment of COPD.[15] We surmised that 3,4-diaminocyclobut-3-ene-1,2-dione would be a good bioisostere for this urea based on its previous use as a cyanoguanidine replacement by researchers at Wyeth.[16]

We prepared a series of cyclobutenediones in a fashion similar to that exemplified for compound **19** in Scheme 1. The corresponding cyclobutenedione analog, **1**, of GSK's urea in Figure 1 was a potent inhibitor of CXCR2 binding with an IC_{50} of 0.036 μM.[17] Further investigation of the left side aniline, Table 1, revealed that the aniline NH and hydroxyl were critical since substitution or removal of these groups, as in compounds **2** and **3**, resulted in dramatic loss of potency. Removal of an electron withdrawing group reduced potency by 10-fold as in the des-nitro compound, **4**, but shifting the nitro group from position 4- to position 5- had no impact on binding activity, **5**. Replacement of the nitro group at either the 4- or 5- position with other electron withdrawing groups such as cyano was well tolerated, **6** and **7**. Carboxylic acid was not well tolerated at either position 3- or 4-, as shown in compounds **8** and **9**. However, installation of methyl ester at position 3- resulted in a more potent compound **10**. Based on this result, the methyl ester was replaced with a series of simple amides, **11**–**15**. The dimethylamide **15** was found to have the best potency within this series. When the dimethylamide

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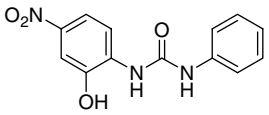
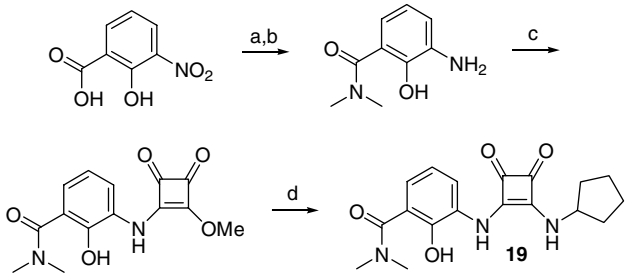


Figure 1. GSK urea for CXCR2.

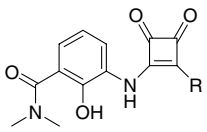


Scheme 1. Reagents and conditions: (a) 2 M dimethylamine in THF, PyBroP, DIEA, DCM; (b) H_2, 5%Pd on C, CH_3OH; (c) 3,4-dimethoxy-3-cyclobutene-1,2-dione, CH_3OH; (d) cyclopentylamine, DIEA, CH_3OH, 60 °C.

aniline was further substituted with halide, potency was maintained as exemplified with compound **16**.

With dimethylamide aniline as the optimized left side, we prepared a series of cyclobutenediones to explore the right-hand side SAR, Table 2. The right side NH was found to be critical since substitution with methyl, **17**, resulted in dramatic loss of potency. Since the electron-rich aniline moiety is well-known to have an unfavorable metabolic profile,[18] we chose to focus on alkyl replacements. Introduction of cyclohexyl and cyclopentyl replacements for aniline provided potent compounds **18** and **19**. Introduction of a heteroatom as in **20** or a fused ring system as in **21** resulted in significant loss of

Table 2. Effect of right side substituents on CXCR2 binding versus IL-8



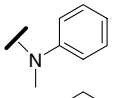
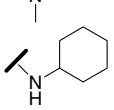
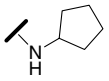
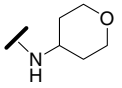
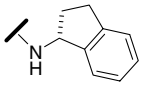
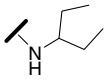
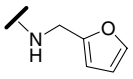
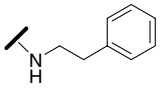
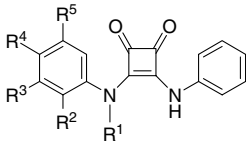
Compounds	R	CXCR2 IC_{50} (μM)
17		7.200
18		0.010
19		0.008
20		0.171
21		0.548
22		0.005
23		0.010
24		0.014
25		0.041
26		0.926

Table 1. Effect of aniline substituents on CXCR2 binding versus IL-8



Compounds	R^1	R^2	R^3	R^4	R^5	CXCR2 IC_{50} (μM)
1	H	OH	H	NO2	H	0.036
2	Me	OH	H	H	H	28.6
3	H	H	H	NO2	H	8.6
4	H	OH	H	H	H	0.476
5	H	OH	H	H	NO2	0.056
6	H	OH	H	H	CN	0.074
7	H	OH	H	CN	H	0.003
8	H	OH	H	CO2H	H	3.1
9	H	OH	CO2H	H	H	1.0
10	H	OH	CO2Me	H	H	0.053
11	H	OH	CONH2	H	H	0.034
12	H	OH	CONHMe	H	H	0.022
13	H	OH	CONHEt	H	H	0.065
14	H	OH	CONHCH2Ph	H	H	0.139
15	H	OH	CONMe2	H	H	0.002
16	H	OH	CONMe2	H	Cl	0.002

Table 3. In vitro chemotaxis, Caco2, and liver microsome results for selected compounds (data shown are means ± standard deviation of two or more measurements)

Compounds	CXCR2 chemotaxis IC_{50} (μM)	Caco2 Papp A:B (nm/s) (predicted absorption)	Human microsome (% remaining)	Rat microsome (% remaining)
15	0.026 ± 0.011	24 ± 1.7 (low)	64 ± 2	67 ± 7
19	0.1448 ± 0.0002	101 ± 4.6 (high)	72 ± 3	56 ± 5
22	0.0191 ± 0.0099	120 ± 2.4 (high)	82 ± 5	75 ± 7

potency. Simple acyclic alkyls were found to be as good as cyclic alkyls, as exemplified by 2-ethylpropyl, isopropyl, and *tert*-butyl compounds **22**, **23**, and **24.** While unbranched heterobenzylics such as furanyl compound **25** showed significant potency, further chain elongation to phenethyl resulted in a much weaker compound, **26**.

Three potent compounds, **15**, **19**, and **22**, were selected for further evaluation in chemotaxis[19], Caco2[20], and rat and human liver microsome assays,[21] Table 3. All three compounds were potent inhibitors of CXCR2-mediated chemotaxis. Although compound **15** was the most potent (chemotaxis $IC_{50} = 0.026 \pm 0.011$ μM), its predicted absorption based on Caco2 permeability was low. This compound and others with right-side anilines also had poor apparent solubility in most solvents. Cyclobutenediones with right-side alkyls, such as **19** and **22**, had much better apparent solubility and had high predicted absorption based on Caco2 results. The ethylpropyl compound **22** was a potent inhibitor of chemotaxis ($IC_{50} = 0.0191 \pm 0.0099$ μM), predicted to be well-absorbed based on Caco2 results, and predicted to have good metabolic stability based on human and rat microsome studies (>50% remaining after 30 min at 37 °C). These findings justify further SAR development and in vivo studies.

In summary, we have developed a potent series of cyclobutenedione CXCR2 receptor antagonists. Preliminary pharmacokinetic studies suggest that this type of compound is amenable to further drug development. Additional developments and in vivo results have been reported[22] and will appear in a subsequent publication.

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17. CXCR2 binding assay: For each 200 μl reaction, a working mixture of 0.020 μg/μl hCXCR2-CHO over-expressing membranes with a specific activity of 0.6 pmol/mg (Biosignal, Montreal [Quebec], Canada) and 2 μg/μl wheatgerm-agglutinin (WGA) coated SPA beads (Amersham Biosciences, Piscataway, NJ) was prepared in CXCR2 assay buffer (25 mM Hepes, pH 7.4, 2.0 mM

$CaCl_2$, and 1.0 mM $MgCl_2$) (Sigma, St. Louis, MO). This mixture was incubated on ice for 5 min. A 0.40 nM stock of ligand, [^{125}I]IL-8 (NEN Life Sciences Products, Inc., Boston, MA), was prepared in the CXCR2 assay buffer. Test compounds were first serial diluted by half-log dilutions in DMSO (Sigma) and then diluted 8.3-fold in CXCR2 assay buffer. The above solutions were added to a Corning #3604 NBS (non-binding surface) 96-well assay plate as follows: 50 μl test compound or 12% DMSO, 100 μl of membrane and SPA bead mixture (Final [membrane] = 2.0 μg/reaction; Final [SPA bead] = 200 μg/reaction), and 50 μl [^{125}I]IL-8 (Final [IL-8] = 0.10 nM). The assay plates were incubated for 2 h at room temperature. Binding of [^{125}I]IL-8 to the bead/membrane mixture was detected using a Perkin Elmer–Wallac Microbeta 1450 liquid scintillation counter. The data were fit to a one-site competition binding model for IC_{50} determination using the program GraphPad Prism (GraphPad Software, Inc., San Diego, CA). All IC_{50}s represent the average of two or more determinations and the standard deviations were no greater than 50% from the mean.

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19. CXCR2 chemotaxis assay: test compounds were dissolved in 100% DMSO and diluted by half-logs. The compounds were subsequently diluted 100× in assay medium to bring the DMSO concentration to 1.0%. 6.6 μl of test compound was then mixed with 60 μl hCXCR2-CHO cells. The lower chamber of a ChemoTx plate (Neuroprobe, Gaithersburg, ND) was filled with 30 μl of 18 nM IL-8 (R&D Systems, Minneapolis, MN). The empty upper chambers were affixed to the lower plate and 25 μl of 1.5E6 hCXCR2-CHO cells, pre-incubated for 100 min with compound, was added to the upper well. Migration proceeded for 40 min at 37 °C in a humidified incubator with 5% CO_2. After removing non-migrated cells from the top of the plate, migrated cells were quantified by adding 25 μl CellTiter Glo™ (Promega) for 10 min and then reading fluorescence on a Wallac Victor. Maximum chemotactic response was determined by cells to which no compound was added (positive control), whereas the negative control (unstimulated) was defined by the absence of IL-8 in the lower chamber.
20. Caco2 assay: Caco2 assays were conducted using BD BioCoat™ HTS Caco-2 Assay System provided by BD Sciences (Bedford, MA) according to the manufacturer's instructions.
21. Microsome assay: microsome stability studies using human and rat microsomes were conducted as follows: test compounds (1 μM final concentration) were incubated for 30 min at 37 °C in a mixture containing 100 mM potassium phosphate buffer (pH 7.4), 1 mM β-NADPH (Sigma, St. Louis, MO), 0.4% bovine serum albumin (Intergen, Purchase, NY), and human or rat liver microsomes (BD Gentest, Bedford, MA) with a final P450 content of 300 nM in a total volume of 0.5 ml. The reaction was terminated by the addition of 0.1 ml methanol containing 0.003 mg/ml ketanserin (Sigma) as internal standard (IS) and followed by addition of 0.75 ml ethyl acetate for liquid extraction. After a 10-min vortex and 10-min centrifugation at 20,000*g*, 0.5 ml of the ethyl acetate phase was removed and evaporated under a nitrogen stream. The residue was reconstituted in 0.1 ml methanol/H_2O (1:1) solution and subjected to HPLC-MS analysis. The percentage of compound remaining was calculated by taking the percentage of the average peak area of 30-min samples from the average peak area of the corresponding 0-min samples.
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