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Identification and structure–activity relationships of 1-aryl-3-piperidin-4-yl-urea derivatives as CXCR3 receptor antagonists

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Abstract—The synthesis and biological evaluation of a series of 1-aryl-3-piperidin-4-yl-urea derivatives as small-molecule CXCR3 antagonists is described. SAR studies resulted in significant improvement of potency and physicochemical properties and established the key pharmacophore of the series, and led to the identification of **9t**, which exhibits an IC_{50} of 16 nM in the GTPγS^{35} functional assay.

The chemokine receptor CXCR3 has been of interest for a number of years as a potential target for treatment of inflammatory diseases such as multiple sclerosis,[1] asthma[2] and rheumatoid arthritis.[3] CXCR3 belongs to the superfamily of seven transmembrane spanning G-protein-coupled receptors (GPCRs) and is predominantly expressed by activated T cells, NK cells and B cells. It binds to three members of the CXC chemokine family: MIG (CXCL9), IP-10 (CXCL10) and I-TAC (CXCL11) resulting in receptor activation and cellular chemotaxis. Antagonising CXCR3 may result in decreased trafficking of activated Th1 effector cells and memory T cells to sites of inflammation. CXCR3 may also be involved in the retention of activated lymphocytes within inflamed tissues following activation with MIG, IP-10 or I-TAC. Blocking strategies have demonstrated efficacy in several murine disease models including arthritis,[4] transplant rejection,[5] diabetes[6] and inflammatory bowel disease.[7] Small-molecule CXCR3 antagonists have been reported in the literature,[8] including T487 **1**[8a] and TAK779 **2**[8b] (which blocks CXCR3 as well as CCR2 and CCR5[9]) (see Fig. 1).

In order to identify hit compounds for our CXCR3 project, a screen of 15,000 compounds was carried out using a FLIPr-based calcium flux assay. The compounds were selected for their hit-like molecular properties and similarities to known GPCR ligands. From this screen, several hit compounds were identified with potency in the micromolar range, including the piperidine urea **3**, which was chosen as a starting point for a hit to lead medicinal chemistry programme. Importantly this compound provided excellent potency against both the human *and* murine receptors (Table 1), demonstrating that cross-species activity is considerably more achievable in this system than has been reported in development of antagonists of CCR1–3[10] (see Fig. 2).

In order to support the medicinal chemistry project, a radiolabelled GTPγS^{35} assay was developed,[11] which reconfirmed the HTS hits.

As shown in Table 1, there remained significant challenges in developing **3** into a lead-like antagonist series. The compounds showed poor aqueous solubility, high log *D* and potent CYP2D6 inhibitory activity. Nevertheless, the structure was ideally suited to rapid analogue generation using solution-phase parallel synthesis, and detailed investigation of the template was carried out with the goal of identifying potent and drug-like molecules for lead optimisation.

Initial variation of the aryl urea moiety was carried out on 0.1 mmol scale, coupling the aminopiperidine **4**[12] with a set of 48 commercially available isocyanates to give ureas **5** (Scheme 1). The final compounds were obtained by adding the crude reaction mixtures to Oasis

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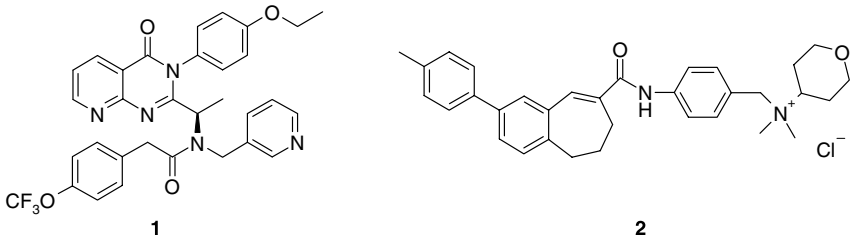


Figure 1. CXCR3 antagonists.

Table 1. Properties of compound **3**

hCXCR$_3$ K_i (μM)[a]	mCXCR$_3$ K_i (μM)[a]	CYP 2D6 (μM)	Aq sol (mg/ml)[b]	log *D*	hMic (μg/ml/min)
0.110	0.40	0.6	0.001	4.95	72

[a] GTPγS^{35} assay.[11]
[b] At pH 6.5.

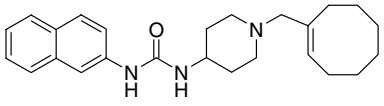
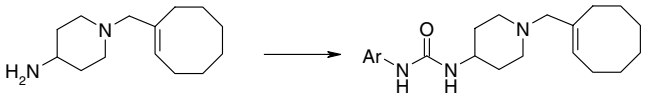


Figure 2. CXCR3 hit structure.



Scheme 1. Reagents: ArNCO, CH_2Cl_2, 60–85%.

MCX acidic ion exchange cartridges. Washing with ethyl acetate, methanol and water to remove unwanted by-products; then elution with methanolic ammonia gave the products as solids with purity greater than 95%. As shown in Table 2, a broad range of aromatic groups gave potent CXCR3 antagonists, the most active being the 3,5-bis-trifluoromethyl urea **5f** with a K_i of 36 nM. However, the most potent compounds retained log *D* > 4 and poor aqueous solubility, an exception being **5e** which provided improved properties at the expense of affinity for the receptor.

Table 2. hCXCR3 K_i values for ureas **5** in GTPγS^{35} functional assay

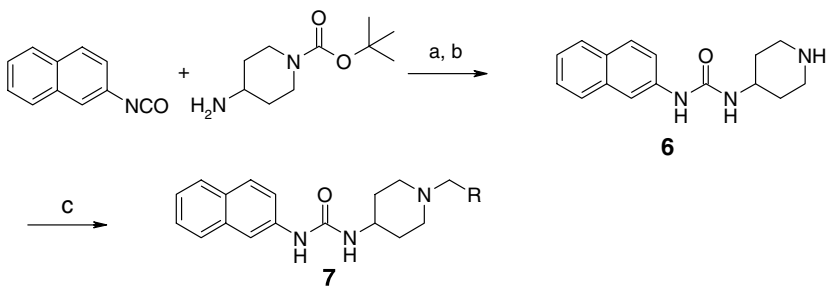
Compound	Ar	K_i[a] (nM)
3	2-Naphthyl	110
5a	1-Naphthyl	IA
5b	3-(Trifluoromethyl)phenyl	88
5c	3-Chlorophenyl	201
5d	3-(Trifluoromethoxy)phenyl	104
5e	3-Acetylphenyl	637
5f	3,5-Bis(trifluoromethy)lphenyl	36
5g	3-Fluoro-5-(trifluoromethyl)phenyl	47
5h	3,5-Dichlorophenyl	41
5i	4-Phenoxyphenyl	306
5j	3-Cyanophenyl	IA
5k	4-Cyanophenyl	IA
5l	4-*tert*-Butylphenyl	1040
5m	4-(2,6-Dichloropyridyl)	3400
5n	Thien-3-yl	IA
5o	3-Methoxycarbonylphenyl	660
5p	2-(5-Phenylthienyl)	108

[a] IA <50% at 10 μM.

Investigation of the right-hand side cycloaliphatic group was carried out by reductive amination of the piperidine **6** with a range of aldehydes chosen to explore the steric requirements for CXCR3 potency (Scheme 2).

As shown in Table 3, CXCR3 activity in this template is highly dependent on the nature of the right-hand side group, with only the myrtenyl derivative **7h** giving potency comparable to **3**. It was striking that the cyclooctyl derivative **7d** was inactive. A range of benzylic groups such as **7f** were included in the set, none of which gave measurable antagonism of the receptor.

Compound **7h** was further characterised and found, unsurprisingly, to have a similarly high log *D* and poor



Scheme 2. Reagents: (a) CH_2Cl_2; (b) 1 N HCl in Et_2O, 86% over two steps; (c) RCHO, $NaBH(OAc)_3$, trimethyl orthoformate/CH_2Cl_2 (1:1), 40–65%.

Table 3. hCXCR3 K_i values for ureas **7** in GTPγS[35] functional assay

Compound	R	K_i[a] (nM)
3	Cyclooctene	110
7a	Cyclohexyl	IA
7b	1-Norbornene	IA
7c	1-Cyclohexene	IA
7d	Cyclooctane	IA
7e	2,4,4-Trimethylpentane	IA
7f	3,4-Dichlorophenyl	IA
7g	(2,6,6-Trimethylcyclohex-1-enyl)methyl	IA
7h	(−)Myrtenyl	290
7i	*R*(+)-4-Isopropylcyclohexen-1-yl	IA
7j	Phenylmethyl	1300
7k	Phenylethyl	2200

[a] IA <50% at 10 μM.

solubility to compound **3**, with the cycloaliphatic group having a strong contribution to the lipophilicity of the template.

Alteration of the central 4-aminopiperidine core was investigated and it was found that changes such as replacement with 3-aminopyrrolidine, 3-aminoazetidine, 3-aminomethylmorpholine, piperazine and homopiperazine (Fig. 3) all resulted in significant loss of potency. Subsequent work therefore continued to focus on the 4-aminopiperidine template.

In order to further investigate the SAR of the left-hand side aromatic group, particularly towards improving the drug-like properties, the myrtenylpiperidine **8** was prepared and reacted with a series of commercially available arylisocyanates. The synthetic approach to the more elaborated ureas was carried out either through the coupling of a constructed isocyanate with amine **8** (Scheme 3; **9o**, **9p**, and **9q**) or through palladium cross-couplings with a common arylbromide (Scheme 4; **9i**, **9j**, and **9k**).

In exploring the aryl SAR for the myrtenyl template, the 3,5-bis(trifluoromethyl)phenyl (**9b**) was again found to give potent inhibitory activity as was the 3-fluoro-5-trifluoromethylphenyl derivative **9t**, which was found to have a K_i against murine CXCR3 of 227 nM. The regioisomeric derivatives **9s** and **9u** were significantly less active. Polar substituents were included with the aim of identifying potent antagonists with tractable physicochemical properties. The 3-methoxycarbonyl phenyl compound **9v** was moderately active (376 nM), but hydrolysis to the carboxylic acid **9w** or reduction to the hydroxymethyl derivative **9x** gave inactive

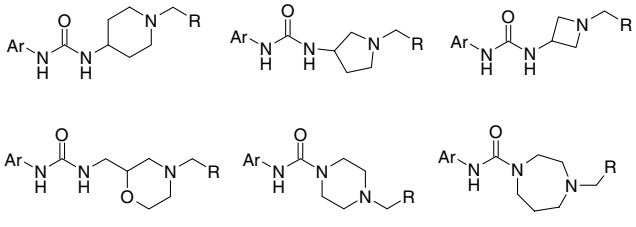
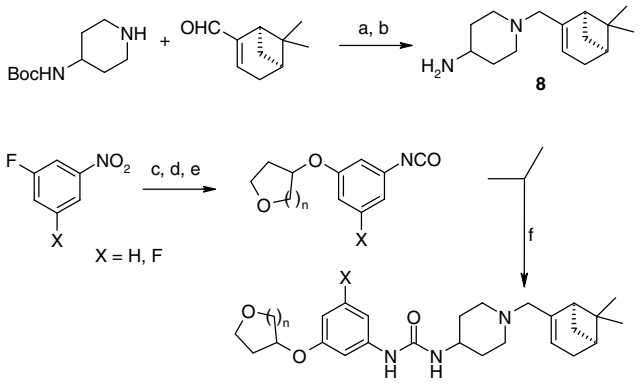
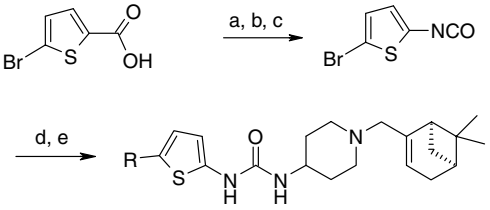


Figure 3. Piperidine replacements investigated.

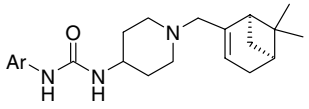


Scheme 3. Reagents and conditions: (a) $NaBH(OAc)_3$, trimethyl orthoformate/CH_2Cl_2 (1:1), 76%; (b) 1 N HCl in Et_2O; (c) ROH, NaH, DMF, 55–70%; (d) H_2 Pd/C, EtOH, 95%; (e) triphosgene, DiPEA, CH_2Cl_2, 0 °C, quant.; (f) CH_2Cl_2, quant.



Scheme 4. Reagents and conditions: (a) $(COCl)_2$, cat. DMF, CH_2Cl_2; (b) NaN_3, acetone/water, 0 °C; (c) Δ, PhMe, 82% over three steps; (d) **8**, CH_2Cl_2, 78%; (e) $RB(OH)_2$, $Pd(PPh_3)_4$, aq Na_2CO_3, THF, reflux, 35–62%.

Table 4. hCXCR3 K_i values for ureas **9** in GTPγS[35] functional assay



9

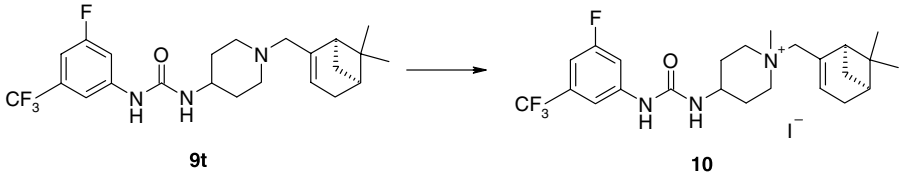
Compound	Ar	K_i[a] (nM)
9a	Phenyl	2000
9b	3,5-Bis(trifluoromethyl)phenyl	73
9c	3-Acetylphenyl	258
9d	3,5-Difluorophenyl	171
9e	3-Methoxyphenyl	429
9f	3-Trifluoromethoxyphenyl	31
9g	4-Trifluoromethoxyphenyl	175
9h	5-Phenylthien-2-yl	191
9i	5-(4-Trifluoromethylphenyl)thien-2-yl	39
9j	5-(3-Methoxyphenyl)thien-2-yl	29
9k	5-(2-Chlorophenyl)thien-2-yl	46
9l	5-Bromopyridin-3-yl	171
9m	5-(Thien-2-yl)pyridin-3-yl	70
9n	5-(Thien-3-yl)pyridin-3-yl	107
9o	3-(Tetrahydrofuran-3-yloxy)phenyl	141
9p	3-(Tetrahydrofuran-3-yloxy)-5-fluorophenyl	83
9q	3-(Tetrahydropyran-4-yloxy)phenyl	191
9r	3-(Morpholin-4-ylmethyl)phenyl	IA
9s	2-Fluoro-5-trifluoromethylphenyl	229
9t	3-Fluoro-5-trifluoromethylphenyl	16
9u	4-Fluoro-5-trifluoromethylphenyl	81
9v	3-Methoxycarbonylphenyl	376
9w	3-Hydroxycarbonylphenyl	IA
9x	3-Hydroxymethylphenyl	IA

[a] IA <50% at 10 μM.

Table 5. hCXCR3 K_i values, physicochemical properties and PK data for selected compounds

Compound	K_i (nM)	Solubility[a] (μg/ml)	log *D*	CL_{int}[b] (μL/min/mg protein)	CL_p[c] (mL/min/kg)
3	110	0.1	4.95	226	nt
9m	70	4	3.47	324	112
9o	141	927	3.87	287	222
9p	83	874	3.00	97	nt
9t	16	23	4.21	67	nt
10	28	55	2.35	66	34.5

nt, not tested.
[a] At pH 6.5.
[b] Mouse microsomal clearance at 0.5 μM.
[c] Mouse clearance. Compounds dosed 1 mg/kg iv.



Scheme 5. Reagents: MeI, Et_2O.

compounds. We found that the 3-methoxy derivative **9e** gave modest potency (429 nM); however, modulation to the 3-tetrahydrofuran ether **9o** significantly improved activity (141 nM). Addition of a 5-fluoro substituent gave **9p** which combined good potency (83 nM) with much improved physicochemical properties (log *D* 3.0).

Phenylthiophene **9h** was found to be active and further derivatives **9i**, **9j** and **9k** gave excellent potency but poor physicochemical properties. In order to improve this, the *meta*-bromopyridine **9l** was prepared from 5-bromonicotinic acid with identical reagents and conditions as in Scheme 4 to give **9m**. The log *D* of this compound was found to be 3.47, which demonstrated that incorporation of more hydrophilic aromatic groups can significantly improve the physicochemical properties whilst retaining good potency (see Table 4).

As this chemical series was providing a good range of affinities and promising initial physicochemical properties, we evaluated selected compounds in preliminary DMPK.

Although the compounds have moderate to high microsomal clearance, the solubility and log *D* were significantly improved compared to compound **3** (Table 5).

Quaternary ammonium salts have been reported as potent chemokine receptor antagonists (e.g., compound **2**). For this reason, we investigated the effect of quaternisation in this series. Compound **9t** was treated with methyl iodide to give **10** as a mixture of *cis*/*trans* isomers (see Scheme 5).

Compound **10** gave good potency, log *D* and solubility (Table 5). It gave an excellent potency in an ITAC-driven chemotaxis assay (EC_{50} of 5 nM).[13] However, due to poor $CaCO_2$ permeability and low oral absorption, quaternisation was not pursued further.

In summary, a new series of small-molecule non-quaternary CXCR3 antagonists has been discovered. SAR studies resulted in significant improvement in potency, and established the key requirements for affinity in the series. These findings provide a structural CXCR3 template for the further optimisation of potency and pharmacokinetic properties. We will report on these findings in due course.

Acknowledgment

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11. $GTP\gamma S^{35}$ binding was determined using CHO-hCXCR3 membranes (Euroscreen; 20 μg/ml), SPA beads (GE Healthcare; 5 mg/ml) in the presence or absence of GDP (10 μM), saponin (50 μg/ml) and $GTP\gamma S^{35}$ (Amersham; 300 pM). Assays were carried out in 96-well plates where membranes and ligand (ITAC/IP10; R&D Systems) were incubated for 60 min (rt) in assay buffer (20 mM Hepes, 100 mM NaCl, 10 mM MgCl, 1 mM EDTA (pH 7.4), and 0.1% BSA) prior to addition of $GTP\gamma S^{35}$. Following a further 2-h incubation at rt, plates were centrifuged and read on a Topcount® (Perkin-Elmer).
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13. IL1.2 cells stably transfected with hCXCR3 were resuspended at a concentration of 1×10^7 cells/ml in RPMI-1640 media containing 0.1% BSA. Fifty microlitre aliquots of these preparations were also pre-incubated with the antagonist of interest at a relevant concentration for 30 min at room temperature prior to the assay being performed. CXCL11 at a fixed concentration of 50 nM was added to the lower chambers of 96-well ChemoTX® microchemotaxis plates (a 5 μm pore diameter, Receptor Technologies, Banbury, UK) supplemented with increasing concentrations of antagonist. The membrane was then put in place and 20 μl of the appropriately treated cells was placed on top of the filter, in duplicate (200,000 cells per well). The chambers were then incubated at 37 °C with 95% CO_2 in a humid environment for 5 h. After incubation, the upper surface of the filter was scraped to remove the unmigrated cells, the filter removed and the cells migrating into the lower chamber were counted on a haemocytometer. Data are shown as the number of cells/high power field and are means of three separate experiments. Vehicle alone (RPMI-1640 media containing 0.1% BSA with 0.5% DMSO) had no effect on the dose-dependent migration of L1.2 hCXCR3 transfectants in response to CXCL11.