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Bioorganic & Medicinal Chemistry Letters 17 (2007) 6806–6810
Bioorganic & Medicinal Chemistry Letters

Development of CXCR3 antagonists. Part 2: Identification of 2-amino(4-piperidinyl)azoles as potent CXCR3 antagonists

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Received 7 September 2007; revised 8 October 2007; accepted 10 October 2007
Available online 17 October 2007

Abstract—Development of a lead series of piperidinylurea CXCR3 antagonists has led to the identification of molecules with alternative linkages which retain good potency. A novel 5-(piperidin-4-yl)amino-1,2,4-thiadiazole derivative was found to have satisfactory in vitro metabolic stability and to be orally bioavailable in mice, giving high plasma concentrations and a half life of 5.4 h.

CXCR3 is a 7-transmembrane G-protein coupled receptor, which is expressed by activated T cells, NK cells, mast cells and B cells. CXCR3 binds three members of the CXC chemokine family: MIG (CXCL9), IP-10 (CXCL10) and I-TAC (CXCL11) resulting in receptor activation and cellular chemotaxis. A number of blocking strategies have demonstrated reductions of disease severity in animal models of arthritis,[1,2] inflammatory bowel disease,[3] diabetes[4] and transplant rejection.[5] Studies in human patients have suggested a role for CXCR3 in rheumatoid arthritis, multiple sclerosis, diabetes, transplant rejection, chronic obstructive pulmonary disease[6] and asthma.[1] CXCR3 is thus an attractive target for the development of anti-inflammatory therapies, and a number of groups have published approaches to the development of small molecule antagonists.[7]

In our previous publication,[8] we described the identification of the naphthyl urea **1a** by high throughput screening of a compound set selected as being 'GPCR-friendly'. The hit molecule formed the basis of early hit to lead activity leading to the identification of molecules such as **1b** with improved physicochemical properties and excellent potency (Fig. 1).

Within this series, modification of physicochemical properties by introduction of solubilising aryl substituents could be achieved, leading to examples with improved characteristics.[8] Nevertheless, we had found that the series as a whole tended to have high log *D*, low solubility and poor in vivo pharmacokinetics. There were, therefore, significant challenges in developing drug-like molecules, which could be used as tools with which to explore the potential of CXCR3 antagonism in models of human disease.

Our strategy in further development was to examine the effect of modification of the urea linkage, so as to establish whether a robust alternative chemical series could be generated.

As reported previously, we had found that replacing the lipophilic myrtenyl right hand side lost potency and it was therefore retained for this phase of the series development.

Our first investigation was around *N*-substituted urea derivatives **4** and **5**, to assess the importance of the urea in binding of the molecules. These compounds were constructed from the myrtenylpiperidine **2**[8] as shown in Scheme 1. *N*-Alkyl amines **3** were generated by acylation of **2** under basic conditions, followed by reduction with lithium aluminium hydride. The *N*-alkyl ureas **4** were then obtained in good yields by reaction with 3-trifluoromethyl phenyl isocyanate.

Keywords: CXCR3 antagonist; (Piperidinyl)azoles; Benzazole; Optimisation; Pharmacokinetics.
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doi:10.1016/j.bmcl.2007.10.029

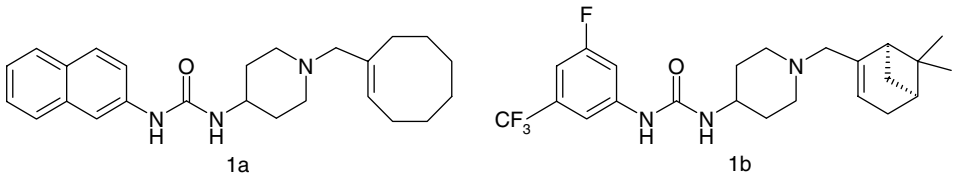
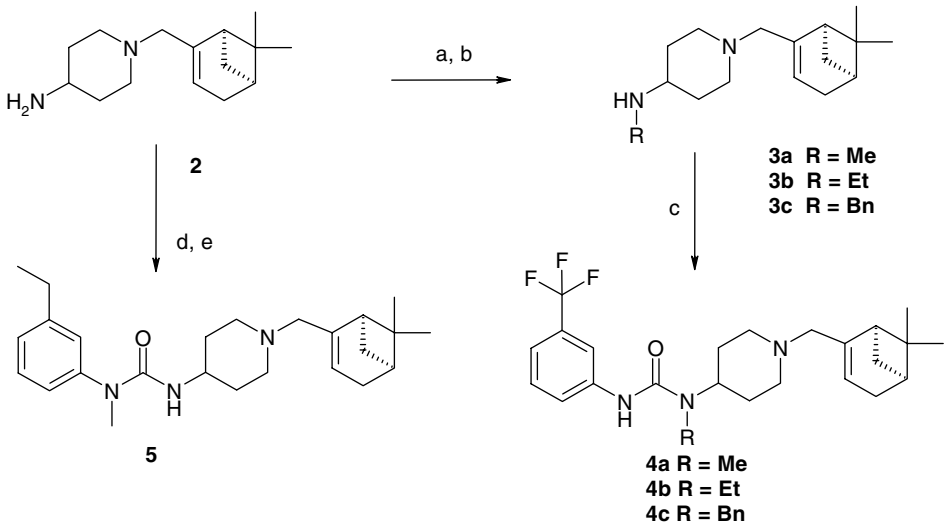


Figure 1. Hit molecules.



Scheme 1. Reagents and conditions: (a) RCOCl, Et_3N, DCM, 75–90%; (b) LAH, THF, reflux, 85%; (c) 3-trifluoromethylphenyl isocyanate, DCM, 57–85%; (d) triphosgene, Hunig's base, DCM; (e) 3-ethyl *N*-methyl aniline, 43% over two steps.

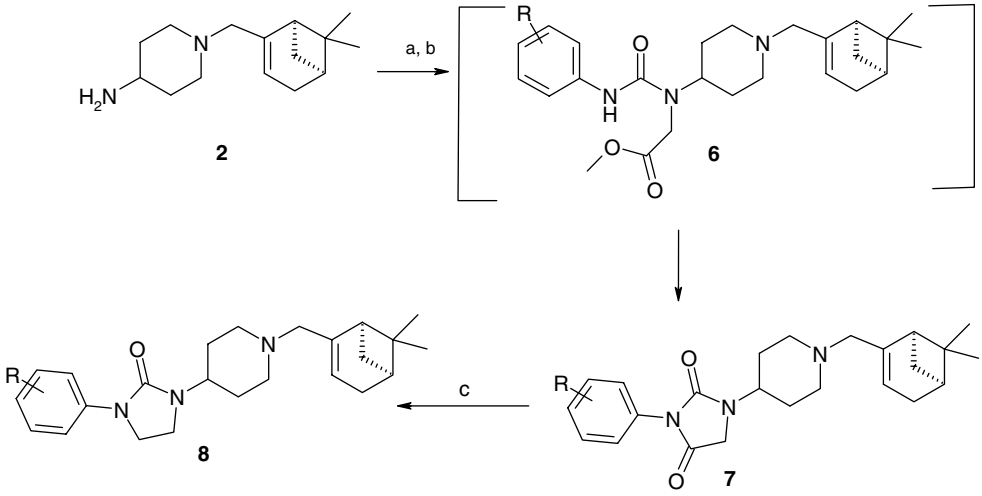
Table 1. Urea modifications

Compound	hCXCR3 K_i (μM)
1b	0.016
4a	0.18
4b	3.6
4c	IA
5	3.5

K_i's measured in ITAC stimulated GTPγS assay using CXCR3 transfected CHO membranes and are the average of two values.[8] IA = <20% inhibition at 10 μM.

To access the 1-methyl urea **5**, the amine **2** was converted into the required isocyanate by reaction with triphosgene and coupled with 3-ethyl *N*-methyl aniline in modest yield.

As shown in Table 1, these modifications to the urea moiety resulted in a loss of potency when compared to **1b**. Methylation of the nitrogen adjacent to the piperidine ring was tolerated, but larger groups resulted in a dramatic loss of potency. Likewise, methylation adjacent



Scheme 2. Reagents and conditions: (a) methyl bromoacetate, DMF, K_2CO_3, 65%; (b) ArNCO, DCM, 70%; (c) $NaBH_4$, TFA, 45%.

Table 2. Hydantoins and imidazolinones

Compound	R	hCXCR3 K_i (μM)	log *D*	CL_{INT}[a] (μL/min/mg)
7a	3,5-$(CF_3)_2$	0.026	>5.5	220
7b	3-F, 5-CF_3	0.10	4.85	290
7c	3-Et	0.38	4.7	190
7d	3-S-Me	0.75	5.0	230
7e	3-CF_3	0.22	4.6	318
7f	3-CF_3, 4-Cl	0.12	5.3	280
7g	3-COMe	IA	3.4	ND
8a	3,5-$(CF_3)_2$	0.17	>5.5	7
8b	3-F, 5-CF_3	0.20	4.6	88

K_i measurements are an average of two determinations. IA = <20% inhibition at 10 μM. log *D* measured by octanol-water partition at pH 7.4.

[a] Murine microsomal clearance at 0.5 μM.

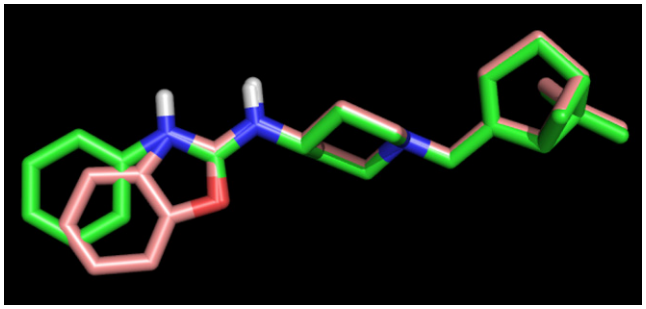


Figure 2. Overlay of aryl urea (green carbons) with benzoxazole (pink carbons).

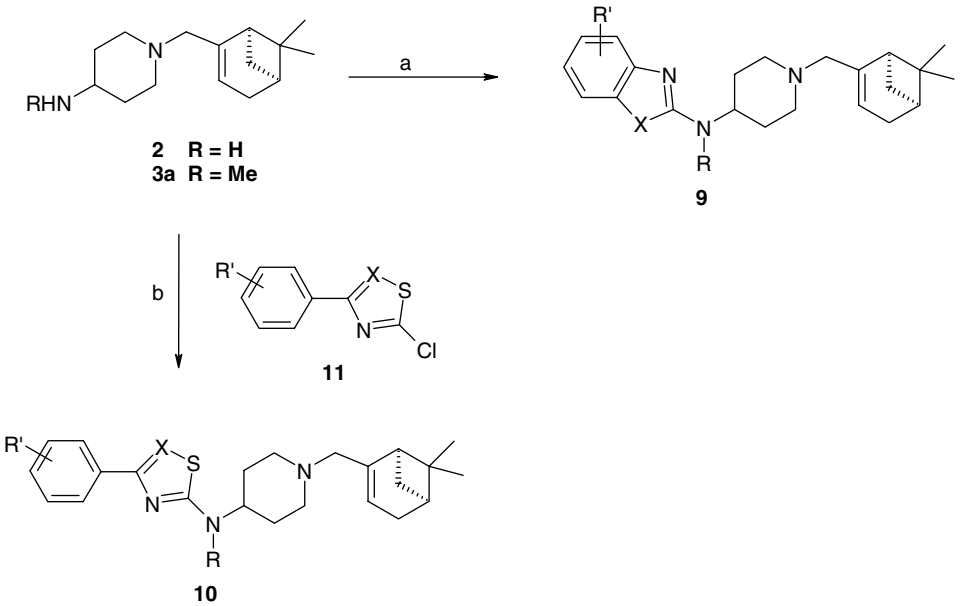
to the aryl group was highly detrimental to activity, suggesting that the urea NH in this position plays an important role in binding. A number of urea replacements such as thioureas and carbamates were also investigated (not shown) and found to result in reduced activity.

We wished to investigate whether polar substituents attached to the urea linker could lead to additional binding interactions, thus restoring potency, and we attempted to prepare the ester **6** as shown in Scheme 2. However, the main product after HPLC purification was in fact the hydantoin **7a**. Having previously established that urea substitution adjacent to the aromatic ring was not tolerated, we were surprised to find that **7a** is a potent receptor antagonist with K_i of 26 nM. A set of close analogues was prepared in which changes to the aromatic substitution were investigated. As shown in Table 2, a range of aromatic substituents provided active compounds although all were less potent than **7a**. The ketone **7g** demonstrated reduced log *D*, but at the expense of potency.

The imidazolinones **8a** and **8b** were also generated, by reduction of the hydantoins with sodium borohydride in the presence of trifluoroacetic acid.

The hydantoin compounds tested were highly lipophilic and proved to be very highly metabolized in vitro by murine microsomes. In addition, some hydantoins were found to be hydrolytically labile, opening up under aqueous conditions. While we were encouraged by the profile of the imidazolinone **8a** which gave good potency and microsomal stability we found that the compound had very poor aqueous solubility and that close analogues such as **8b** were more highly turned over in the microsomal stability assay. We therefore felt that the template would be difficult to adapt to provide combined potency and drug-like properties, however the work demonstrated the principle that cyclic linkers could serve to replace the urea.

We therefore set out to incorporate heteroaromatic replacements for the hydantoin moieties with the aim of improving both the physicochemical properties and stability of the products.



Scheme 3. Preparation of aminopiperidinyl benzazoles and aryl azoles. Reagents and conditions: (a) 2-chlorobenzazole, NMP, Et_3N, 140 °C, 23–45%; (b) Et_3N, NMP, 100 °C, 40–55%.

Table 3. Benzazole compounds

Compound	X	R′	R	hCXCR3 K_i (μM)	log *D*	CYP 2D6 (μM)	CL_{INT}[a] (μL/min/mg)
9a	O	H	H	0.68	4.7	ND	ND
9b	O	H	Me	0.72	4.6	ND	ND
9c	S	H	H	0.35	>5	13	1
9d	S	H	Me	0.14	>5	2.2	78
9e	NH	H	Me	0.95	4.2	ND	ND
9f	S	4-Cl	Me	0.46	5	ND	ND
9g	S	5-Cl	Me	0.29	5	3.5	41
9h	S	6-Cl	Me	0.17	5	7.3	48
9i	S	7-Cl	Me	0.62	>5	4.1	ND
9j	S	4-Br	H	0.15	>5	1.2	140
9k	S	6-Br	H	0.13	>5	0.7	150
10a	CH	H	H	0.88	4.7	IA	ND
10b	CH	3,4-di-Cl	H	0.46	>5	IA	ND
10c	CH	3,5-$(CF_3)_2$	H	0.26	>5	IA	34
10d	N	3,5-$(CF_3)_2$	H	0.27	5	15	16

K_i measurements are a mean of two determinations. ND, not determined. IA = <20% at 50 μM.
[a] Murine microsomal clearance at 0.5 μM.

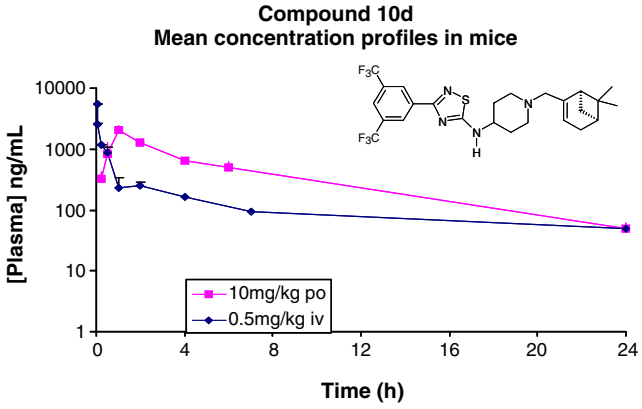


Figure 3. In vivo pharmacokinetics of compound **10d**.

Comparison of an aryl urea with a benzoxazole using the PyMol program[9] showed a close overlay. Both molecules displayed the piperidine in a similar orientation, the azole ring nitrogen occupying the same position as one of the urea nitrogens and the aromatic rings in similar orientation (Fig. 2).

We chose to construct a series of benzazole and aryl azole derivatives, with a view to evaluating the effect of substitution on the aromatic ring and on the nitrogen linker.

A set of commercially available 2-chlorobenzazoles was assembled and it was coupled in parallel by heating under microwave conditions with amines **2** and **3a**. The crude products were purified directly by preparative HPLC of the reaction mixtures with no work-up required, thus allowing rapid SAR generation.

Similarly, a set of aryl-substituted aminoazoles **10** was constructed by nucleophilic substitution of chloroazoles **11**[10] with **2** under basic conditions (Scheme 3).

As shown in Table 3, moderate potency was achieved with the benzoxazoles **9a** and **9b**. The benzothiazole **9c** appeared to give increased potency and low microsomal clearance. Activity was further improved by methylation of the linker to give **9d**, although this change also led to increased microsomal clearance and CYP inhibition. The benzimidazole **9e** was less potent than either benzoxazole or benzothiazole derivatives and was not investigated further.

A series of chlorobenzothiazoles showed that the 6-substituted derivative **9h** was equipotent to the unsubstituted analogue **9d**, while the 7-chloro compound **9i** was less potent. The 4 and 6-bromobenzothiazoles gave good activity, but were potent CYP2D6 inhibitors and were also highly metabolized in the microsome incubation assay.

The aryl azoles **10** gave moderate CXCR3 activity, the most potent examples bearing the familiar 3,5-*bis*-trifluoromethyl substitution pattern. Although **10d** was found to be highly lipophilic, it exhibited good in vitro microsomal stability and showed only weak inhibition of cytochrome P450 enzymes. This compound was taken forward into a PK study to establish whether its in vitro stability would result in satisfactory in vivo exposure.

As shown in Figure 3 and Table 4, compound **10d** was orally bioavailable with a long plasma half-life and low clearance of 2 mL/min/kg, thus demonstrating that the template could deliver non-urea CXCR3 antagonists with improved PK. This compound had human plasma

Table 4. In vivo pharmacokinetics of compound **10d**

Compound	cMax (ng/ml)	AUC (ng h/ml)	$T_{1/2}$ (h)	CL_p (ml/min/kg)	log *D*	Sol (μg/ml)
10d	2012	10,800	5.4	2.8	5	2.7

protein binding of 99.7% and was found to have activity of 1 μM in a murine CXCR3 assay.[8]

In conclusion, we have developed a novel series of potent CXCR3 antagonists based on a piperidinyl-azole scaffold, suitable for further optimization. Further development of this series and in vivo characterization of resulting molecules are the subject of future publications.

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