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Development of CXCR3 antagonists. Part 3: Tropenyl and homotropenyl-piperidine urea derivatives

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Abstract—The optimization of a series of 1-aryl-3-piperidinyl urea derivatives is described in which incorporation of tropenyl and homotropenyl moieties has led to significant improvements in activity and drug-like properties. Replacement of the central piperidine with an *exo*-tropanyl unit led to the identification of compound **15** which provides a combination of excellent potency against human and murine receptors, drug-like properties and pharmacokinetics, thus providing a valuable tool for the evaluation of CXCR3 antagonists in models of human disease.

The chemokine receptor CXCR3 binds and is activated by the interferon γ-inducible chemokines, CXCL9, CXCL10 and CXCL11, leading to cellular activation and chemotaxis.[1,2] The receptor and its ligands have been implicated in human diseases including multiple sclerosis,[3] arthritis,[4] IBD,[5] asthma,[6] COPD[7] and transplant rejection.[8]

A number of groups have reported the development of small molecule CXCR3 antagonists,[9] although only Amgen's AMG487 has so far progressed into clinical trials.[10] This compound has been reported to have efficacy in a murine bleomycin-induced cell recruitment assay.[9a]

In our previous publications,[11] we have described the development of the screening hit **1a** into potent urea **1b** and arylazole **1c** derivatives. In the development of these molecules we found that the bulky cycloalkenyl moiety was important for potency; simpler cycloaliphatic rings and aryl groups in this position gave rise to much reduced potency. The cycloalkene brought with it a contribution to high log *D*, often resulting in low solubility and limited metabolic stability. We therefore felt that there was an opportunity to further improve our series by modifying this part of the template.

Our goal was to provide a series of potent and drug-like molecules suitable for use in vivo to elucidate the potential of CXCR3 antagonists in inflammatory diseases. In this paper, we describe the identification of replacements for the cycloalkene which result in dramatically improved physicochemical and DMPK characteristics and potency (Fig. 1).

In order to examine the cycloaliphatic area of the molecule, we selected a set of 100 heteroatom-containing carboxylic acids and aldehydes based on similarity in shape and volume to our commonly used myrtenyl right hand side according to Verloop parameters.

Preparation of the substituted ureas **3** was performed as shown in Scheme 1. The bis-trifluoromethyl urea **2** was coupled with selected carboxylic acids, followed by reduction with lithium aluminium hydride; or reacted with aldehydes in the presence of sodium triacetoxyborohydride. In general, the products were found to be inactive, however the cyclic sulfone **3a** and the piperidines **3b** and **3c** were found to have interesting activity.

We were particularly intrigued that the modestly active sulfonyl derivative **3a** showed a dramatic improvement

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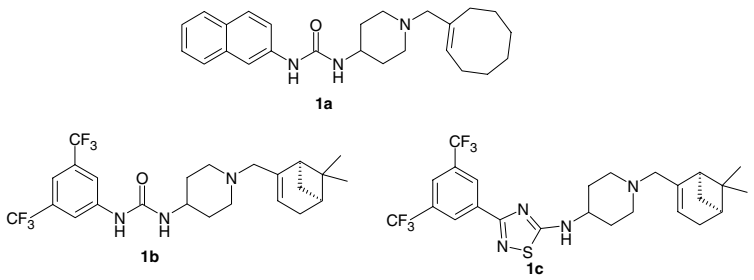
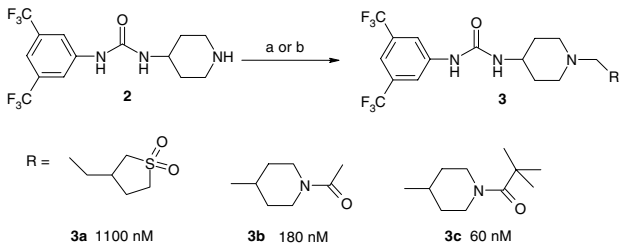


Figure 1. Hit molecules.



Scheme 1. Reagents and condition: (a) i—RCO_2H, HOBT, EDC, DMF, 50–85%; ii—LAH, THF, reflux; (b) RCHO, $NaBH(OAc)_3$, DCE, 4A molecular sieves.

in physicochemical properties and metabolic stability compared to the myrtenyl analogue **1b** (Table 1). The properties translated into good pharmacokinetics following oral dosing to mice, with **3a** showing 52% bioavailability and a plasma half-life of 5.5 h. The acetylpiperidine **3b** was more active and again showed significantly improved properties over **1b** and potency was further improved in the pivaloyl derivative **3c**, though at the expense of both physicochemical properties and metabolic stability. These observations gave us confidence that, by suitable replacement of the right hand cycloalkene, we could significantly improve the physicochemical properties in this series while retaining potency.

While the overall profiles of the acylpiperidine derivatives were encouraging, they were generally less potent than equivalent myrtenyl derivatives, and modelling showed that acyl piperidines and the myrtenyl ring system occupied quite different volumes, the latter appearing more globular (Fig. 2). We reasoned that modification of the piperidine ring could restore potency.

Table 1. Comparison of potency and properties of compounds **1a** and **3a–c**

Compound	hCXCR3 K_i[a] (μM)	Log *D*	Sol[b] (μg/ml)	CL_{INT}[c] (μL/min/mg)	PPB
1b	0.026	5.1	<1	82	99.9
3a	1.1	2.8	1360	3.5	97
3b	0.18	3.1	850	4.5	95
3c	0.06	4.2	45	80	99

[a] $GTP\gamma S^{35}$ assay. Results are an average of two values.[11]
[b] At pH 6.5.
[c] Human microsomal clearance at 0.5 μM. Log *D* was measured by octanol–water partition at pH 7.4.

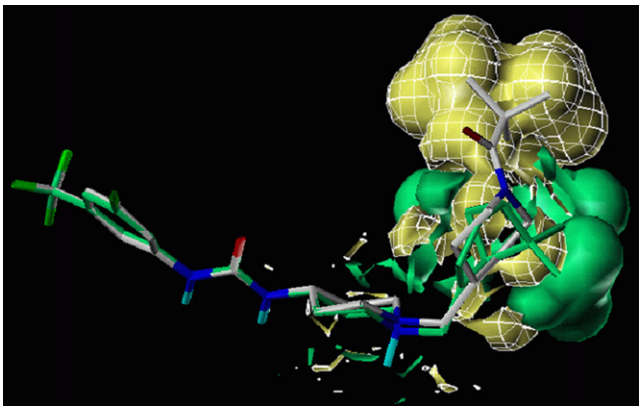


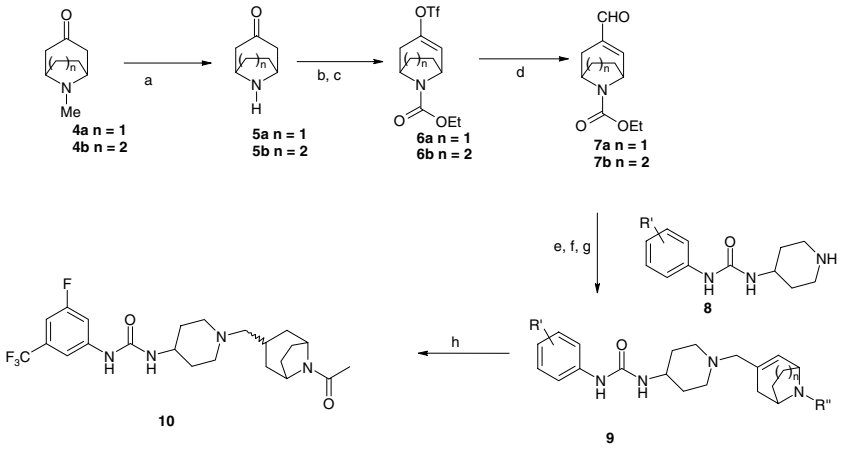
Figure 2. Overlay of compound **3c** (gold) and myrtenyl analogue (green) carried out using Sybyl (Tripos).

We found that methylation at the 2, 3 and 4-positions around the piperidine ring resulted in reduced potency (not shown), and instead targeted bridged piperidine derivatives, which appeared to fill a similar volume to the myrtenyl group. We therefore selected tropanyl and homotropanyl derivatives for synthesis.

Construction of the required aldehydes was carried out from commercially available *N*-methyl tropanone **4a** and *N*-methyl homotropanone **4b** (Scheme 2). These were N-demethylated and converted to carbamates, then into their enol triflates **6** under standard conditions. The aldehydes **7** were prepared in one step by carbonylation in the presence of triethyl silane.[12] These were coupled with the urea building block **8** and the intermediate alkenyl products **9** deprotected and acylated in good overall yields. Hydrogenation of the tropene derivative **9b** gave **10** as a one-to-one mixture of *endo* and *exo* isomers.

The tropene derivatives **9b–9e** gave good potency against the human receptor as shown in Table 2. Increasing the size of the terminal acyl group improved activity at the expense of increased log *D* and impaired metabolic stability. The *N*-acetyl homotropene **9f** gave excellent activity against both human and murine receptors. In this case, the pivaloyl compound **9g** offered no advantage over **9f**, giving significantly increased log *D* and microsomal instability with no compensating increase in potency.

We had previously found that favourable changes to physicochemical properties could be achieved by incor-



Scheme 2. Reagents and conditions: (a) 1-chloroethylchloroformate, DCE, 80%; (b) EtOCOCl, Et_3N, DCM, 100%; (c) LiHMDS, THF, Tf_2NPh, 90%; (d) CO, Et_3SiH, DMF, $Pd(Oac)_2$, dppp, *i*-Pr_2NEt 75%; (e) $NaBH(OAc)_3$, DCM, 4A molecular sieves, 65–80%; (f) TMSI, CH_3CN, reflux, 96%; (g) RCOCl, Et_3N, DCM; (h) H_2, Pd/C, MeOH, 86%.

Table 2. Tropene and homotropene derivatives

Compound	R′	R″	*n*	Log *D*	CL_{INT} (μL/min/mg)	K_i (μM) human	K_i (μM) mouse
9a	3-F, 5-CF_3	CO_2Et	1	4.6	48	0.19	0.95
9b	3-F, 5-CF_3	COMe	1	3.0	11	0.027	0.12
9c	3-F, 5-CF_3	CO-*i*-Pr	1	3.9	58	0.028	0.18
9d	3-F, 5-CF_3	CO-*c*-Pr	1	3.4	9	0.020	0.08
9e	3-F, 5-CF_3	CO-*t*-Bu	1	4.2	60	0.018	0.10
9f	**3-F, 5-CF_3**	**COMe**	**2**	**3.4**	**9**	**0.009** ± 3.2 (*n* = 5)	**0.038** ± 15.6 (*n* = 4)
9g	3-F, 5-CF_3	CO-*t*-Bu	2	4.5	74	0.010	0.057
9h	3-COMe	COMe	2	2.8	10	0.28	ND
9i	3-O-*i*-Pr	COMe	2	2.5	22	0.011	0.033
9j	3-F, 5-O-*i*-Pr	COMe	2	2.7	50	0.003	0.006
10		COMe	1	2.8	0	0.12	0.65
15		**COMe**	**2**	**3.1**	**7**	**0.007** ± 2.9 (*n* = 5)	**0.006** ± 1.3 (*n* = 5)

n refers to the bridge position (Scheme 2).

poration of polar aromatic substituents[11] and found that such modifications were tolerated in the piperidine–homotropene template. For example, the 3-acetyl derivative **9h** gave moderate activity while the isopropyl ether **9i** provided similar potency to **9f** against both human and murine receptor, along with a reduction in log *D*. A further improvement in potency was achieved with the 3-fluoro-5-isopropoxy derivative **9j**. On the basis of these results, we concluded that the homotropene moiety provided a binding motif, which could tolerate a range of aromatic substitutions and contributed to a drug-like profile.

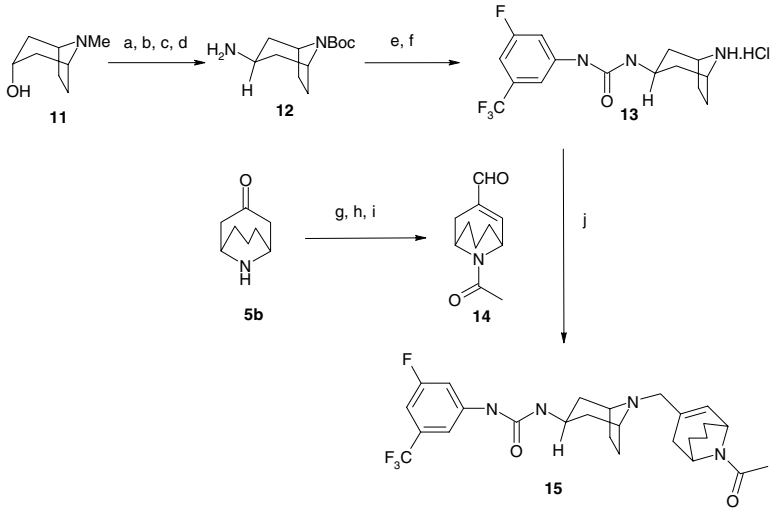
In metabolite identification studies on **9f** we observed several sites of oxidation around the central piperidine ring. We rationalized that bridging or substituting this moiety could protect from metabolism and further improve the overall profile of the series. Modelling suggested that an *exo*-tropanyl unit could be accommodated and this was synthesized as shown in Scheme 3.

Commercially available tropine **11** was reacted with phthalimide under Mitsunobu conditions. The product was demethylated and protected as its *N*-Boc derivative and the phthalimide group cleaved by hydrazinolysis to give the aminotropane **12**. This was coupled with 3-fluoro, 5-trifluoromethylphenyl isocyanate and the product deprotected to give **13** in excellent overall yield.

The *N*-acetyl homotropene aldehyde **14** was constructed in good overall yield using the chemistry described in Scheme 2. Reductive amination was efficiently achieved using a titanium tetraisopropoxide mediated imine formation followed by reduction with sodium triacetoxyborohydride,[13] providing a convergent synthesis suitable for the preparation of multigram quantities of **15**.

Compound **15** provided similar potency against the human receptor compared with **9f**, with an improvement in activity against murine CXCR3. Both log *D* and microsomal stability were in the desired range and compounds **9f** and **15** were compared to determine their suitability as tools for in vivo pharmacology.

Compounds **9f** and **15** were orally dosed to Balb/c mice at 30 mg/kg, and both compounds demonstrated excellent bioavailability with **15** giving sustained plasma concentrations greater than 3 times its plasma pA2 value[14] beyond the 12 h time point (Fig. 3). In order to translate exposure to a pharmacological effect a pharmacodynamic readout was also obtained using the murine



Scheme 3. Reactions and conditions: (a) Phthalimide, diisopropylazodicarboxylate, PPh_3, DCM, 75%; (b) 1-chloroethyl chloroformate, DCE, 87%; (c) Boc_2O, DCM, Et_3N, 95%; (d) hydrazine, ethanol; (e) 3-fluoro, 5-trifluoromethyl phenyl isocyanate, DCM, Et_3N, 90%; (f) HCl, ether, MeOH, 95%; (g) AcCl, Et_3N, DCM, 93%; (h) LiHMDS, Tf_2NPh, THF, 90%; (i) CO, Et_3SiH, $Pd(OAc)_2$, dppp, DMF, 78%; (j) $Ti(OiPr)_4$, THF, $NaBH(OAc)_3$, 75%.

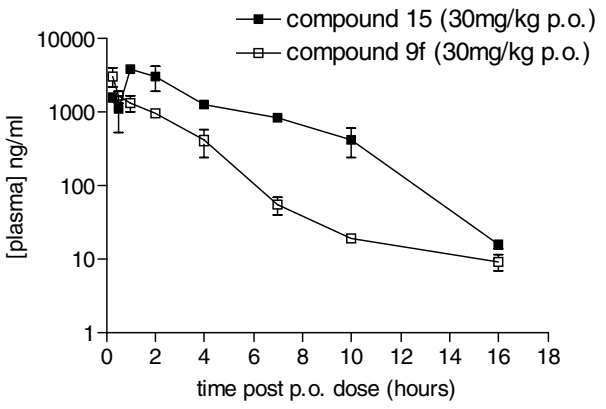


Figure 3. Pharmacokinetics of compounds **9f** and **15** following oral dosing to Balb/c mice at 30 mg/kg.

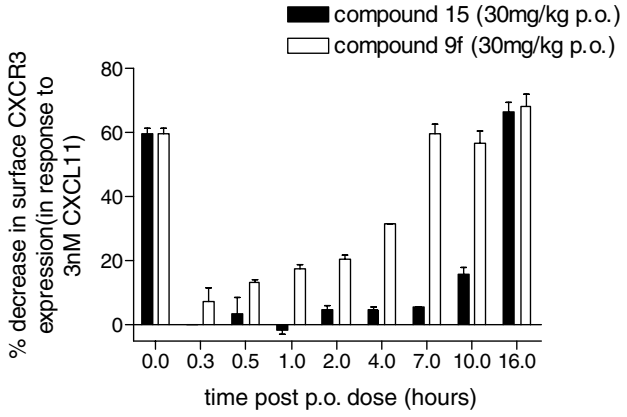


Figure 4. Ex vivo inhibition of murine CXCR3 receptor internalisation, response to 3 nM CXCL11, following administration of compounds **9f** and **15** to mice (30 mg/lg po).

CXCR3 receptor internalisation assay (Fig. 4).[15,16] Compound **9f** demonstrated antagonism of the CXCR3 receptor internalisation response up to 4 h post dose however this declined by 7 h post dose, while the superior pharmacokinetics of compound **15** translated into antagonism of CXCR3 receptor internalisation at 10 h post dose (Fig. 4). These data demonstrated a good correlation between exposure and effect and discriminated between the compounds in vivo, allowing us to select **15** for further evaluation.

We found that **15** gave good aqueous solubility, human plasma protein binding of 93% and only weak inhibition of CYPs 3A4 (32 μM) and 2D6 (15 μM). Selectivity against a panel of 50 receptors was determined and K_i values obtained for those targets where the compound gave greater than 50% inhibition at 10 μM.[17] As shown in Table 3, the compound was selective with less than 50% inhibition of 44 out of the 50 tested targets and a minimum 33-fold separation from its activity against the muscarinic M1 receptor.

Table 3. Selectivity profile of compound **15**

Target	K_i (μM)	Fold difference
CXCR3	0.007	NA
M1	0.23	33
M2	0.28	40
M3	1.2	170
$5HT_{1A}$	0.28	40
Na Channel	0.57	81
$5HT_{5A}$	3.0	430

When compared to the profile of our original screening hit **1a** and the latter derivative **1b** it was apparent that improvements in potency, physicochemical properties and DMPK characteristics had been achieved as shown in Table 4.

In conclusion, we have developed a series of highly potent piperidinyl and tropanyl urea CXCR3 antagonists, leading to the identification of compound **15**, which pro-

Table 4. Comparison of compounds **1a**, **1b** and **15** showing improvements in potency and properties

Compound	hCXCR3 (μM)	mCXCR3 (μM)	Sol (μg/ml)	Log *D*	CL_{INT} (μL/min/mg)	CYP 2D6 (μM)	PPB (%)	% F
1a	0.110	0.40	0.1	4.95	72	0.6	>99.9	1
1b	0.07	0.40	0.7	5.1	105	5	99.7	ND
15	0.007	0.006	40	3.1	7	15	93	70
Fold change **1a**–**15**	16	67	400	1.85 log units	10	25		70

vides an excellent tool for the elucidation of the role of CXCR3 in models of human disease.

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14. In vitro affinity estimates for compounds **9f** and **15** were generated using the murine CXCR3 receptor internalisation assay in the presence of naive mouse plasma. Briefly, activated murine T cells expressing high surface levels of CXCR3 were incubated with 90% plasma, plus agonist (CXCL11) concentrations (0.3–300 nM) and a single antagonist concentration to generate significant rightward shift of the control concentration effect curve. Incubation occurred for 60 min at 37 °C after which time, surface CXCR3 levels were measured by flow cytometry. pA_2 values were 6.73 ± 0.04 for compound **9f** and 7.02 ± 0.16 for compound **15**.
15. Activated murine T cells were incubated with plasma isolated from mice at each timepoint post oral dose with **9f** or **15**. The samples were then stimulated with a single agonist concentration (3 nM CXCL11 = A_{75} in this example) for 60 min at 37 °C after which time agonist was removed and the level of surface CXCR3 was measured by flow cytometry.
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