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Development of CXCR3 antagonists. Part 4: Discovery of 2-amino-(4-tropinyl)quinolines

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Abstract—The synthesis and biological evaluation of a novel series of 2-aminoquinoline substituted piperidines and tropanes incorporating a homotropene moiety is herein described. The series exhibits potent antagonism of the CXCR3 receptor and superior physicochemical properties. Compound **24d** was found to be orally bioavailable, and PK/PD studies suggested it as a suitable tool for studying the role of CXCR3 in models of disease.

CXCR3 is a chemokine receptor which is mainly expressed on CD4+ and CD8+ T cells with a Th_1 phenotype, and in addition, is also expressed on B cells, natural killer (NK) cells, malignant T cells and astrocytes. Interaction occurs with the three chemokines, MIG (CXCL9), IP-10 (CXCL10) and ITAC (CXCL11), which are induced primarily by IFN-γ and are produced by macrophages and other cell types at sites of inflammation. Antagonism of this receptor has shown reduction of disease severity in animal models of arthritis,[1,2] IBD,[3] diabetes[4] and transplant rejection.[5] Studies in human patients have implicated CXCR3 in rheumatoid arthritis, multiple sclerosis, diabetes, transplant rejection and COPD,[6] whilst the receptor has also been shown to drive chemotaxis of mast cells to airway smooth muscle in asthma.[7]

In our previous publications,[8] we described the development of urea piperidine **1b** (Fig. 1) from the screening hit **1a**. This molecule, incorporating the homotropene amide moiety, showed promising potency, physicochemical properties and pharmacokinetics. Replacement of the central piperidine ring with a tropane was found to further improve the potency and drug-like properties in this series.[8b] We had also found[9] that in the azole derivative **1c** we could replace the aryl urea moiety and retain activity against CXCR3.

Going forward, our goal was to develop a series of non-urea derivatives which matched the potency and properties of compounds such as **1b**. This paper describes the development of very potent and drug-like CXCR3 antagonists from the benzazole template **1c**.

Initial work focused on investigating whether the benefits afforded by the homotropene moiety in **1b** would lead to similar improvements in azole **1c**. Incorporation of the homotropene amide right-hand side to the benzazole template was carried out according to Scheme 1 by the condensation of 2-chlorobenzothiazole with 1-BOC-4-methyl-aminopiperidine to give **2**, followed by cleavage of the BOC group with HCl in MeOH to afford **3**. Reductive alkylation with the BOC-homotropene **4**[8b] afforded **5**, which was converted to the corresponding acetyl amide **6** by subsequent deprotection and acetylation.

Comparison of compound **6** with compounds **1b** and **1c** is illustrated in Table 1 below.

We were encouraged to find that compound **6** gave similar potency compared to **1c** thus demonstrating that the homotropene group was well tolerated in this series whilst having significantly lower log *D*. Overall

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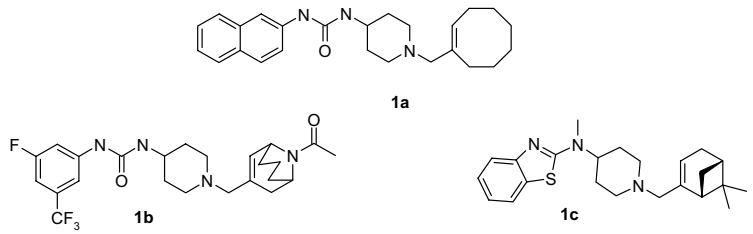
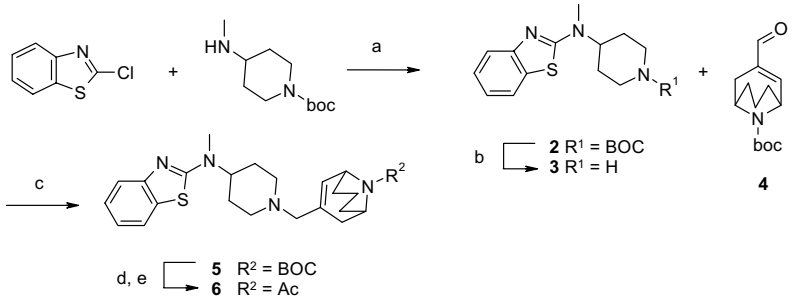


Figure 1. Initial urea and benzazole templates.



Scheme 1. Synthesis of benzazole compounds. Reagents and conditions: (a) neat, 120 °C, 55%; (b) HCl, MeOH, 88%; (c) $NaBH(OAc)_3$, $CH(OMe)_3$, DIPEA, DCM, 65%; (d) HCl, MeOH, 100%; (e) AcCl, DIPEA, DCM, 83%.

Table 1. Comparison of urea template and benzazole templates

Compound	K_i[a] (nM)	log D[b]	Sol[c] (μg/ml)	CL_{INT}[d] (μL/min/mg)	PPB (%)
1b	9	3.4	429	9	98.7
1c	126	>5.5	<1	100	ND
6	95	3.3	ND	26	99.7

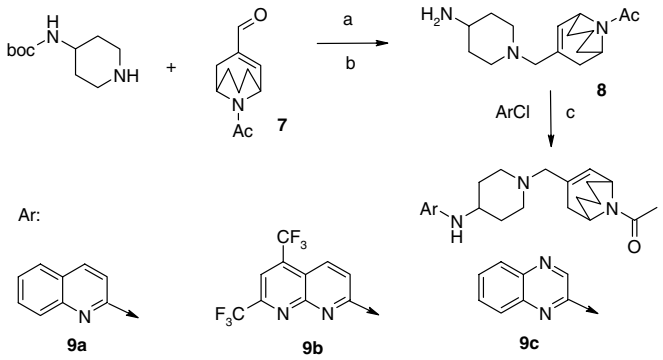
[a] K_i's measured in ITAC stimulated GTPγS[35] assay using CXCR3 transfected CHO membranes and are means of two determinations.[8a]
[b] log D measured by octanol–water partition at pH 7.4.
[c] At pH 6.5.
[d] Human microsomal clearance at 0.5 μM.

properties of **6** were still not ideal however, as indicated for example by the high plasma protein binding value of 99.7%. We felt that substitution of the benzazole template was unlikely to improve this situation markedly, so instead we examined increasing the property space described by this series by combining the piperidine-homotropene motif with a range of alternative 6,6-heterocyclic groups. The aminopiperidine homotropene amide **8** (Scheme 2) was accessed by reductive alkylation of the homotropene aldehyde **7**[8b] with 4-BOC-aminopiperidine followed by deprotection with trifluoroacetic acid. Condensation of commercially available 2-chloroquinoline with compound **8** under microwave irradiation afforded the aminoquinoline **9a** in low yield. Further derivatives were prepared by reaction of **8** with a range of commercial chloroheterocycles. As shown in Table 2, the quinoline **9a** gave potent activity along with good physicochemical properties. Excellent CXCR3 activity was observed with naphthyridine **9b**, indicating that appropriate substitution could dramatically affect potency, whilst the quinoxaline **9c** exhibited a similar profile to **9a**.

The favourable overall profile of **9a** encouraged us to conduct a detailed investigation of substitution on the quinoline template. Substituted 2-chloroquinolines were accessed in a number of ways: compounds **13a** (2,8-dichloroquinoline) and **13b** (2,6-dichloroquinoline) (not drawn) were both commercially available. Compounds **13c–e** (Scheme 3) were prepared by Heck reaction of the corresponding substituted 2-bromoanilines **10c–e** with methyl acrylate followed by acid-mediated cyclisation of the resulting cinnamic acid methyl esters **11c–e** to the corresponding quinolin-2-ones **12c–e**.[10] Chlorination with $POCl_3$ afforded the desired 2-chloroquinolines **13c–e**.

Compound **13f** (Scheme 4) was accessed by alkylation of 7-hydroxyquinoline **15** with 2-iodopropane to give **16**, formation of the *N*-oxide **17** with *m*-CPBA and chlorination with $POCl_3$. Chromatographic separation of the 2-Cl and 4-Cl isomers formed was required.

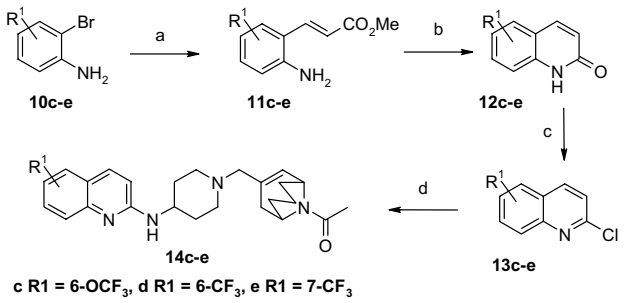
2-Chloroquinolines **13g** and **13h** (Scheme 5) were prepared from 3,5-difluoronitrobenzene **18** by displacement with NaOMe, followed by reduction of the nitro group by hydrogenation to yield **19**. Coupling of the aniline with 3-ethoxyacryloyl chloride according to a literature method[11] afforded the enol ether **20** which was cyclised under acidic conditions to give a mixture of the two possible quinolin-2-ones **21**. Chlorination afforded the 2-chloroquinoline **13g** after chromatographic separation



Scheme 2. Exploring 6,6-bicycles. Reagents and conditions: (a) $NaBH(OAc)_3$, $CH(OMe)_3$, DCM, 78%; (b) TFA, DCM, 100%; (c) DIPEA, NMP, μW, 200 °C, **9a** 28%, **9b** 71%, **9c** 46%.

Table 2. Fused bicycle modifications

Compound	log *D*	K_i (nM)	PPB (%)	CL_{INT} (μL/min/mg)	CYP2D6 (μM)
9a	2.2	135	ND	1	34.5
9b	4.1	15	95.5	18	26.2
9c	2.4	143	ND	2	89.5



Scheme 3. Quinoline synthesis. Reagents and conditions: (a) methyl acrylate, $Pd(OAc)_2$, P(*o*-Tol)$_3$, Et_3N, MeCN, reflux, 27%; (b) HCl, THF/H_2O, reflux, quant.; (c) $POCl_3$, 110 °C, 91%; (d) **8**, $Pd(OAc)_2$, butyl *bis*-1-adamantylphosphine, NaO^{t}Bu, PhMe–NMP, μW 160 °C or heating 80 °C, 69% typical yield.

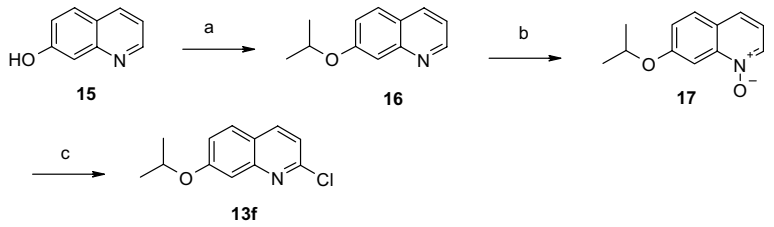
of the two regioisomers. Preparation of **13h** was achieved by BBr_3-mediated demethylation of **13g**, followed by alkylation of the resulting phenol with 2-iodopropane.

The key final step of condensation of the chloroquinolines **13a–h** with the amine **8** to afford **14a–h** was performed in each case according to literature conditions[12] illustrated in the final step of Scheme 3 above. The variable to low yields achieved previously by microwave condensation at 200 °C in NMP (as described in Scheme 2) were improved significantly by the use of these conditions, the best ligand for this reaction being found to be the butyl *bis*-1-adamantylphosphine.[13]

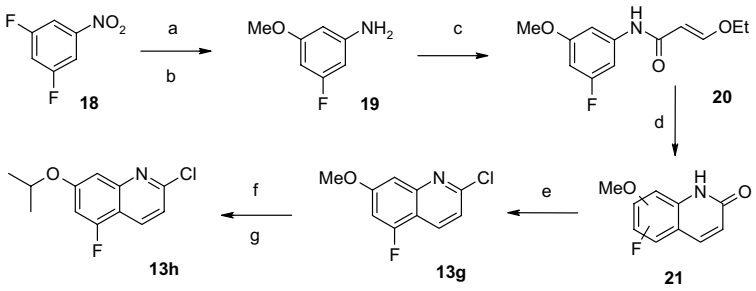
As shown in Table 3, substitution on the quinoline ring-system generally resulted in 10-fold increase in potency compared to **9a**, except at the 8-position (**14a**) this compound being weakly active, presumably due to unfavourable steric interactions. Derivatives **14b**, **14c**, **14d** and **14f** all showed similar potency and properties, although the trifluoromethyl ether **14c** was the most lipophilic (log *D* 3.9). The 7-trifluoromethyl derivative **14e** was the most potent of this series and gave a favourable clearance of 10 μL/min/mg. The disubstituted derivatives **14g** and **14h** showed good potency and properties, but no clear advantage over **14e**.

In our previous publication,[8b] we found that improvements in potency and physicochemical properties could be achieved by incorporating a tropane ring in place of the central piperidine, and this encouraged us to investigate the effect of replacement of the central piperidine with a tropane ring in this series.

Synthesis of the aminotropane homotropene amide building block **23** (Scheme 6) commenced from the known[14] 4-phthalimidotropane **22**. This was reductively alkylated with the aldehyde **7** followed by hydrazinolysis of the phthalimide to afford **23** in good yield. Microwave-assisted condensation with the corresponding



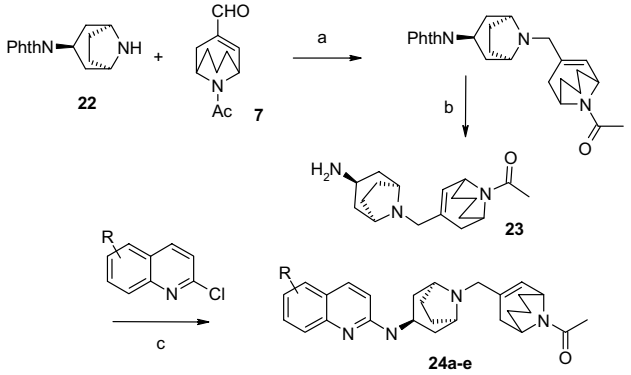
Scheme 4. Chloroquinoline synthesis. Reagents and conditions: (a) ^{i}PrI, Cs_2CO_3, DMF, 100%; (b) *m*-CPBA, DCM, 100%; (c) $POCl_3$, 100 °C, 44% (plus 4-Cl isomer).



Scheme 5. Chloroquinoline synthesis. Reagents and conditions: (a) MeOH, NaH, NMP, 46%; (b) 10% Pd/C, H_2, 79%; (c) EtOCH=CHCOCl, py, DCM, quant.; (d) 75% H_2SO_4, 0 °C, 95%; (e) $POCl_3$, Et_3N, 110 °C, 70% for desired isomer; (f) BBr_3, DCM, 97%; (g) ^{i}PrI, Cs_2CO_3, DMF, 70%.

Table 3. Quinoline ring modifications

Compound	R_1	K_i (nM)	log D	PPB (%)	CL_{INT} (μL/min/mg)
14a	8-Cl	624	ND	ND	ND
14b	6-Cl	26	2.7	95.6	15
14c	6-OCF_3	18	3.9	95.5	23
14d	6-CF_3	21	3.3	95.4	11
14e	7-CF_3	7	2.9	97.3	10
14f	7-O^{i}Pr	25	3.4	ND	ND
14g	5-F-7-OMe	20	3.2	96.3	8
14h	5-F-7-O^{i}Pr	24	3.1	ND	18



Scheme 6. Tropane compounds. Reagents and conditions: (a) $Ti(O^iPr)_4$, $NaBH(OAc)_3$, THF, 100%; (b) N_2H_4, EtOH, 81%; (c) $Pd(OAc)_2$, 2-(dicyclohexylphosphanyl)biphenyl, NaO^{t}Bu, PhMe-NMP, μW, 150 °C, 38–49%.

chloroquinolines as above afforded the desired products **24a**–**e** in moderate yield, the optimum ligand for this step being 2-(dicyclohexylphosphanyl)biphenyl.

We were gratified to find that compounds **24a**–**e** exhibited very potent activity in vitro combined with low intrinsic clearance (Table 4), thus confirming the benefit of changing the central piperidine ring to a tropane. The trifluoromethyl ether **24a** for example was three times more potent than the comparable piperidine derivative **14c**, and benefited from lower log D, PPB and intrinsic clearance values. Compound **24d** was particularly of interest, with a potency of 5 nM and a log D of less than 3.

Table 4. Central tropane modifications

Compound	R	log D	K_i (nM)	PPB (%)	CL_{INT} (mL/min/mg)
24a	6-OCF_3	3.7	6	94.2	12
24b	6-CF_3	3.5	3	98.4	17
24c	7-CF_3	3.6	4	98.4	11
24d	7-O^{i}Pr	2.9	5	96.7	9
24e	5-F-7-OiPr	3.5	6	98.2	12

Compound **24d** was subsequently studied in pharmacokinetic experiments to determine its suitability for dosing in models of inflammatory disease (Fig. 2).

As shown in Figure 2 and Table 5, compound **24d** was orally bioavailable with a long half-life and low clearance of 9 mL/min/kg. We observed dose proportional increases in exposure from 10 to 100 mg/kg and exposure at all doses out to 24 h post dose. In order to translate exposure to CXCR3 receptor blockade, a pharmacodynamic readout was also obtained using the

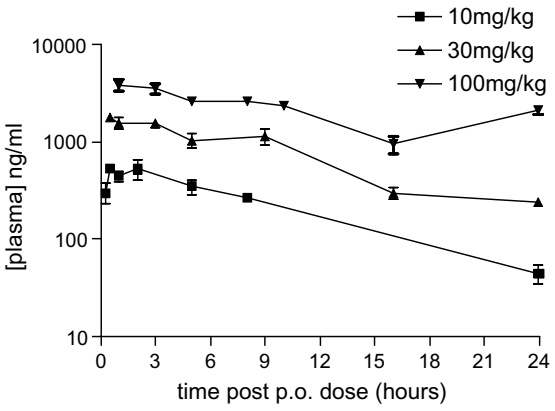


Figure 2. Pharmacokinetics of compound **24d** following oral dosing to Balb/c mice at 10, 30 and 100 mg/kg.

Table 5. In vivo PK of compound **24d at** 30 mg/kg po

Compound	*c*Max ng/mL	AUC (inf) (ng h/mL)	$T_{1/2}$ (h)	CL_p (mL/min/kg)	Sol (pH 6.5) (μg/mL)
24d	1804	21,283	7.6	9	1280

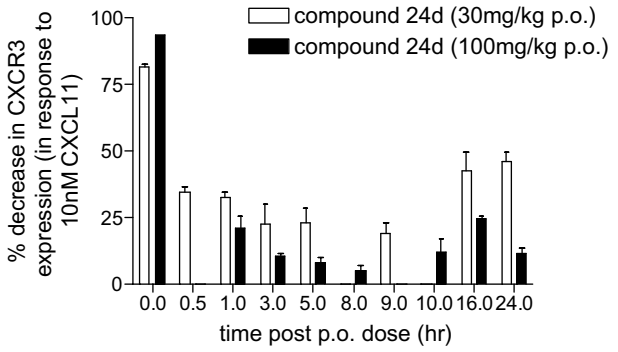


Figure 3. Ex vivo inhibition of murine CXCR3 receptor internalisation response to 10 nM CXCL11, following administration of compound **24d** to mice (30 and 100 mg/kg po).

murine CXCR3 receptor internalisation assay, Figure 3.[8b,15] Following po dosing of 30 mg/kg **24d** complete inhibition of the CXCR3 internalisation response was observed 9 h post dose, with partial inhibition at 16 and 24 h post dose.[16,17] In addition following po dosing of 100 mg/kg **24d** complete inhibition of the CXCR3 internalisation response was observed out to 24 h post dose.[16,17] These data demonstrated a good correlation between exposure and effect and make **24d** suitable for use in the study of CXCR3 antagonism in murine models of disease.

In conclusion, we have developed a series of very potent, orally bioavailable quinoline CXCR3 antagonists with excellent physicochemical properties. Further characterization of these molecules is underway and will be communicated in due course.

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16. An in vitro affinity estimate for compound **24d** was generated using the murine CXCR3 receptor internalisation assay in the presence of naïve 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 value was 7.8 for compound **24d**.
17. Activated murine T cells were incubated with plasma isolated from mice at each timepoint post oral dose with 30 or 100 mg/kg **24d**. The samples were then stimulated with a single agonist concentration (10 nM CXCL11 = A_{86} 30 mg/kg experiment; 10 nM CXCL11 = A_{97} 100 mg/kg experiment) for 60-min at 37 °C. The agonist was removed by washing and the level of surface CXCR3 was measured by flow cytometry.