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The discovery of potent, orally bioavailable pyrimidine-5-carbonitrile-6-alkyl CXCR2 receptor antagonists

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ABSTRACT

A hit-to-lead optimisation programme was carried out on the Novartis archive screening hit, pyrimidine 2-((2,6-dichlorobenzyl)thio)-5-isocyano-6-phenylpyrimidin-4-ol **4**, resulting in the discovery of CXCR2 receptor antagonist 2-((2,3-difluorobenzyl)thio)-6-(2-(hydroxymethyl)cyclopropyl)-5-isocyanopyrimidin-4-ol **24**. The SAR was investigated by systematic variation of the aromatic group at c-6, the linker between c-2 and the halogenated ring, and the c-5 nitrile moiety.

Chemokines are small 8–10 kDa cytokines characterised by four conserved cysteine residues linked via disulfide bonds.[1] Chemokines act through G-protein-coupled receptors (GPCRs) to regulate a variety of effects, including cell migration and inflammatory events. To date, numerous small molecule chemokine antagonists have been described and claimed in patents.[2,3]

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality throughout the world and is a chronic inflammatory disease characterised by neutrophilic inflammation, tissue damage and mucus hypersecretion.[4] There is a significant body of evidence linking the chemokine IL-8 in the pathogenesis of COPD.[5,6] Given the unmet medical need, this has prompted interest in the development of antagonists of the IL-8-activated chemokine receptors CXCR1 and/or CXCR2. The first non-peptide CXCR2 antagonist SB-225002 was described in 1998. Subsequent optimisation led to the CXCR2 antagonist danirixin **1**, which has since progressed into clinical trials for the treatment of COPD.[7]

Several other pharmaceutical companies have disclosed CXCR2 antagonists and amongst these, navarixin **2**,[8] and AZD-5069 **3** (structure not disclosed[9]) are noteworthy of mention. Navarixin progressed into Phase II studies for the treatment of COPD but Merck omitted to list it in their updated development pipeline of January 2012. AZD-5069 recently completed a Phase II trial in COPD patients in 2011[10] and a Phase II trial in bronchiectasis patients in 2012[11] (Fig. 1).

Our previous paper described hit-to-lead efforts for a series of pyrazolo and triazolopyrimidines.[12] In this Letter, we present efforts directed towards a second, chemically distinct hit that led to another series of CXCR2 antagonists.

A HTS was undertaken to identify functional antagonists that blocked the binding of GRO-α to human recombinant CXCR2 (hrCXCR2) expressed in CHO membranes using a [^{35}S]-GTPγS assay. In this assay the amount of accumulated [^{35}S]-GTPγS is directly proportional to the degree of receptor activation. At the hit evaluation stage, compounds with inhibition >50% at10μM were re-confirmed as inhibitors in a 10 point IC_{50} [^{35}S]-GTPγS membrane assay and a more conventional filter wash hrCXCR2 [^{125}I]-GRO-α or [^{125}I]-IL-8 binding assay.[13] A good correlation between the binding and functional assays was noted and binding affinity was not routinely measured during hit-to-lead. On-target activity was confirmed by showing a lack of functional antagonism in a SDF-1 stimulated hrCXCR4 [^{35}S]-GTPγS selectivity assay and periodic spot checking revealed selectivity not to be an issue.

Pyrimidine **4** was discovered from the CXCR2 HTS and although inactive in the functional assay, it displayed a modest level of potency in the binding assay. This activity translated into poor ligand-lipophilicity efficiency (LLE)[14] and the profile of this CXCR2 hit is shown in Table 1.

At the time of investigation, more potent thiazolo[15] and imidazololylpyrimidine[16] structures had been reported. As such,

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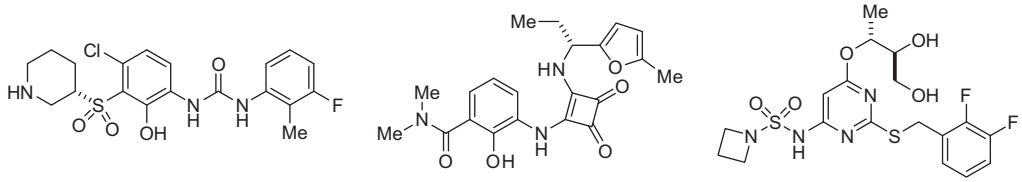
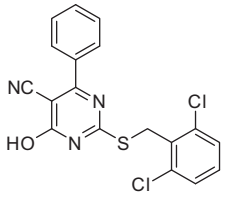


Figure 1. Publicly identified CXCR2 antagonists danirixin and navarixin; compound **3** is believed to be AZD-5069[9].

Table 1
Hit profile of pyrimidine-5-carbonitrile **4**



Measure	Pyimidine **4**
hrCXCR2 [^{125}I]-GRO-α IC_{50}	4.3 μM
[^{35}S]-GTPγS IC_{50}	>30 μM
Molecular weight	388
*c*Log*P*	6.1
Binding LLE (pIC50—*c*Log*P*[a])	−0.7

[a] Compound **4** is weakly acidic and for the purposes of this calculation one can assume Log*P* = Log*D*.

the plan was to examine in sequence the four pyrimidine substituents aiming to improve potency and lower *c*Log*P*. Isosteres and amine substitutions for the pyrimidinol functionality, aromatic/non-aromatic groups at c-6, alternative linkers between c-2 and the halogenated ring and replacements for the c-5 nitrile were explored.

Preference for a thiol linked 2,3-difluorobenzyl had already been noticed elsewhere in a pyrazolo/triazolopyrimidine and thiazolopyrimidine series of antagonists.[12,15] Replacement of the 2,6-dichlorobenzyl with 2,3-difluoro gave compound **5** (Fig. 2) which had measurable activity in the functional assay. This compound was used as a starting point for variation of the other pyrimidine substituents.

As discussed in our previous Letter,[12] there was some concern that pyrimidinol compounds like **5** would have high in vivo clearance following conjugation of the phenol with glucuronic acid. A limited investigation into variation of the phenol at the 4-position was therefore carried out and representative examples of this are shown in Figure 2. Several AZD-5069 analogues have been disclosed containing a hydroxyl or reverse sulfonamide at the 4-position of the pyrimidine ring and the ionisation state of the phenol has been shown to be critical for binding affinity. In our hands, methanesulfonation of **5** to give **6** yielded an inactive compound, consistent with observations from the pyrazolo/triazolopyrimidine series that the presence of larger acidic substituents might interfere with binding to the receptor. Substituting the phenol with an alkyl substituted hydroxylamine also gave an inactive compound **7**. This was an important observation since a series of potent, non-acidic imidazolylpyrimidine antagonists appear to have a similar spatial orientation of functionality.[16]

Having established that the pyrimidinol moiety was important for activity, variation of the 6-phenyl head group was investigated next. Routes to these compounds are shown in Scheme 1. Thiouronium salt **8** was obtained in quantitative yield by treating 2,3-difluorobenzyl bromide with thiourea in ethanol at 65 °C. The synthesis of pyrimidinol **5** and analogues **9**–**21** was accomplished in two steps via Knoevenagel condensation of the aldehyde with ethyl 2-cyanoacetate and reaction of the resulting acrylates [predominantly (*E*)-] with thiouronium salt **8**. Numerous aldehydes were tolerated including electron-withdrawing/electron-rich aromatics and simple aliphatics.

CXCR2 receptor potency of the new 6-phenyl containing ligands was initially guided by the application of the Topliss tree approach with the goal of gaining maximum information from the least number of analogues—a selection of these is shown in Table 2. The Topliss approach takes into account the electronic (σ), lipophilic (π), and steric factors (E_s) for substitution on a phenyl ring

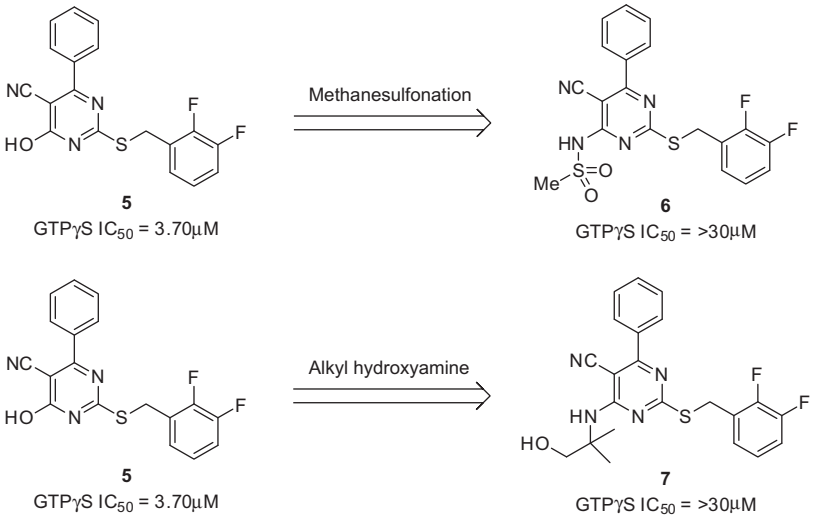
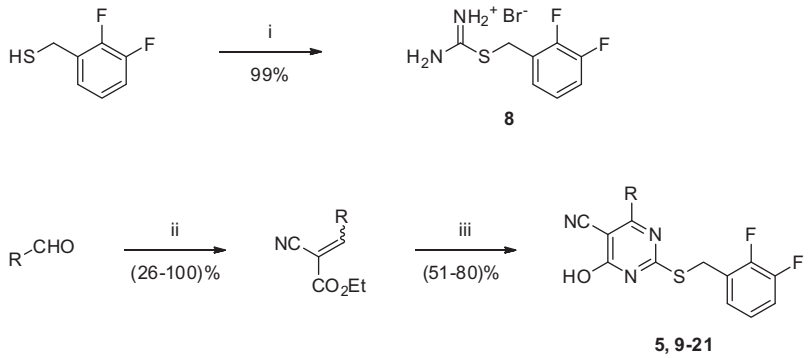
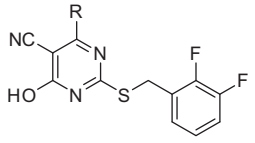


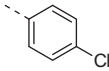
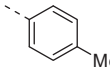
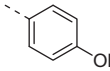
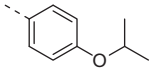
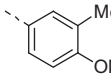
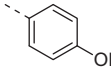
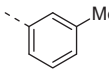
Figure 2. Alternative c-4 groups.



Scheme 1. Reagents and conditions: (i) thiourea, EtOH, 65 °C, 3–4 h; (ii) $NCCH_2CO_2Et$, NH_4OAc, AcOH, toluene, 4 Å molecular sieves, reflux, 3–4 h; (iii) **8**, DIPEA, EtOH, rt.

Table 2
CXCR2 antagonist binding potencies

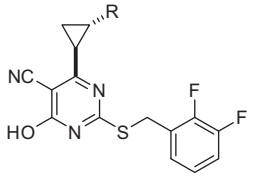


Compound	R	CXCR2 [^{35}S]-GTPγS IC_{50}[a] (μM)	*c*Log*P*	Functional LLE (pIC50−*c*Log*P*[b])
5		3.7	4.9	0.4
9		7.4	5.7	−0.6
10		1.4	5.4	0.5
11		0.71	5.0	1.1
12		1.6	5.8	0
13		4.6	5.5	−0.2
14		0.78	4.5	1.6
15		4.7	5.4	−0.1
16		4.3	3.5	1.9
17		0.90	4.4	1.6
18		4.9	5.0	0.3
19		1.7	4.5	1.3
20		0.14	3.9	3.0
21		3.7	4.8	0.6

[a] Mean of $n \geqslant 2$.
[b] Compounds **5**, **9**–**21** are weakly acidic and for the purposes of this calculation one can assume Log*P* = Log*D*.

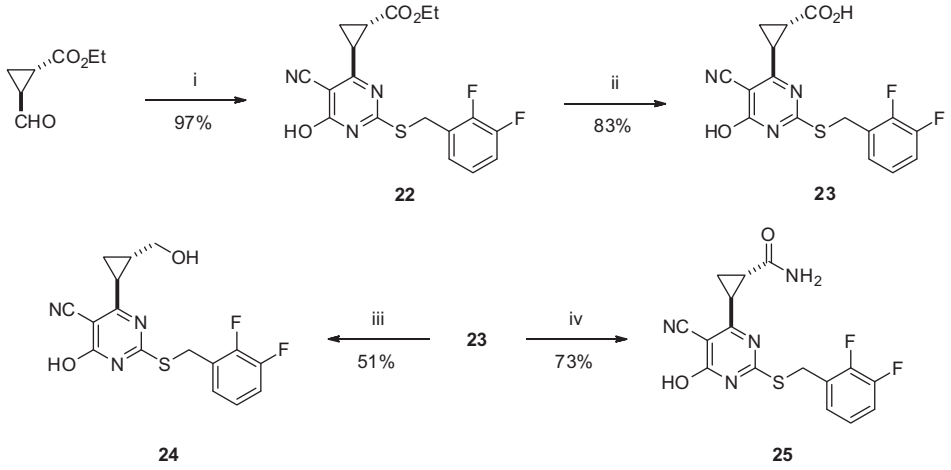
using basic Hansch principles in a non-computerised manner.[17] 4-Chloro substitution of the phenyl ring gave compound **9** of comparable activity to the parent compound **5**. According to the Topliss decision tree, equal potency would most probably result from a favourable $+\pi$ effect counter balanced by an unfavourable $-\sigma$ dependency, whilst lower potency would most likely indicate that activity is $-\sigma$ controlled. Synthesis and comparison of the 4-Me **10** and 4-OMe **11** analogues confirmed that a $-\sigma$ effect was dominant. This prompted follow-on synthesis of 4-O^{i}Pr **12** and 3-Me, 4-OMe **13** in an attempt to further reinforce this effect [the 4-N(Me)$_2$

Table 3
CXCR2 antagonist binding potencies



Compound	R	CXCR2 [^{35}S]-GTPγS IC_{50}[a] (μM)	*c*Log*P*	Functional LLE (pIC50—*c*Log*P*[b])
rac-**22**	CO_2Et	1.5	3.7	2.1
ent-**22a**	CO_2Et	0.32	3.7	2.8
ent-**22b**	CO_2Et	1.3	3.7	2.2
ent-**23a**	CO_2H	0.01	2.8	5.2
ent-**24a**	CH_2OH	0.006	2.4	5.8
ent-**25a**	$CONH_2$	0.007	1.9	6.3

[a] Mean of $n \geqslant 2$.
[b] **22**–**25** are weakly acidic and for the purposes of this calculation one can assume LogP = LogD.



Scheme 2. Reagents and conditions: (i) $NCCH_2CO_2Et$, **8**, K_2CO_3, EtOH, °C, 3–4 h; (ii) 1 M NaOH, EtOH, rt, 18 h; (iii) 1 M BH_3, THF, MeOH, −5 °C (10 min) to rt, 18 h; (iv) HATU, NH_3, Et_3N, DMF, rt, 18 h.

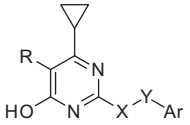
and 4-NH_2 analogues were avoided due to potential for mutagenicity[18]]. No additional increase in activity signalled some π effect suggesting synthesis of the 4-OH **14** and 3-Me **15** analogues. These compounds showed no improvement in activity relative to **11** implying that optimum electronic and hydrophobicity contributions had already been obtained.

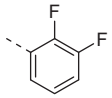
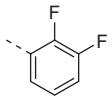
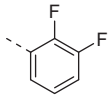
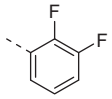
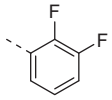
A similar Topliss operational scheme for side-chain alkyl substitution has also been reported.[17] By and large the cases covered are all those other than direct substitution on an aromatic nucleus. We wished to probe the replacement of the 6-phenyl substituent with a 6-alkyl and recognised that the electronic nature of the pyrimidine ring and steric influence of the 5-CN group may complicate application of the approach. Nonetheless, starting with methyl **16** as the base compound, the *iso*-propyl substituent was chosen first on the premise that a $+\pi$ effect was most probable and an increase in activity was obtained with **17**. Cyclopentyl **18** was then synthesised on account of its larger π value with minimal change in steric factor E_s. However, failure of cyclopentyl to show an increase in potency indicated that the optimum π value had already been exceeded. Cyclobutyl **19** and cyclopropyl **20** were made next and gratifyingly, cyclopropyl **20** displayed a significant improvement in activity and LLE relative to **5**. The second possibility of activity increasing with increasing $-\sigma_*$ values was ruled out following synthesis of the *tert*-butyl analogue **21** which displayed comparable activity to cyclopentyl **18**.

SAR studies with a related series of thiazolopyrimidines suggested that a hydroxylated alkyl substituent would be beneficial for potency.[15] With this in mind and using **20** as a starting point, we designed a series of analogues to explore additional parameters of the cyclopropyl motif – a selection of these is shown in Table 3 and the synthesis of these compounds is shown in Scheme 2. Synthesis was accomplished via a one-pot modified Biginelli reaction with (±)-*trans* ethyl 2-formyl-1-cyclopropanecarboxylate and thiouronium salt **8** to give ethyl ester **22**. Alkaline hydrolysis in EtOH gave intermediate acid **23** whilst treatment with borane in THF proceeded smoothly to yield alcohol **24**. HATU mediated coupling of **23** with ammonia in DMF gave carboxamide **25**. In addition to the racemic (±)-*trans*-mixtures generated, compounds **23**–**25** were also isolated as single enantiomers following chiral HPLC purification. Data for the racemate and single enantiomers is presented for compound **22**, whilst data for the most potent enantiomer is shown for compounds **23**–**25**. Confirmation of absolute stereochemistry was not determined at this stage.

Comparison of the racemic ethyl ester *rac*-**22** with the separated enantiomers *ent*-**22a** and *ent*-**22b** revealed that there was a chiral preference for one enantiomer over the other. This was further qualified with analogues **23**–**25**—removal of the ethyl group led to a dramatic increase in potency with acid *ent*-**23a** showing a 30-fold improvement over ethyl ester *ent*-**22a**. Additional manipulation of *ent*-**23a** was well tolerated as exemplified by the

Table 4
CXCR2 antagonist binding potencies



Compound	R	X	Y	Ar	CXCR2 [^{35}S]-GTPγS IC_{50}[a] (μM)
20	CN	S	CH_2		0.14
26	CN	S	CH_2		0.51
27	CN	S	CHMe		4.8
28	CN	S	$C(Me)_2$		6.4
29	CN	CH_2	S		>30
30	CN	CH_2	S		10
31	H	S	CH_2		0.32
32	Br	S	CH_2		1.4
33	I	S	CH_2		3.2

[a] Mean of $n \geqslant 2$.

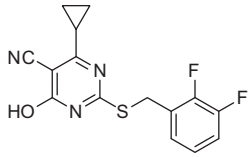
(hydroxymethyl)cyclopropyl derivative *ent*-**24a** and cyclopropanecarboxamide *ent*-**25a**.

As with the pyrazole/triazolopyrimidine series of antagonists,[12] the lipophilic *S*-benzyl was seen as a potential site of metabolic instability and the c-5 nitrile is in an appropriate position to enhance this. As such, a set of alternative linkers at c-2 and replacements for the nitrile at c-5 were synthesised and assessed for potency in comparison to compound **20** (Table 4).

Benzylic substitution may be a strategy for hindering displacement at c-2 but (1-phenylethyl)thio **27** and *gem*-dimethyl **28** were found to be less active than unsubstituted **26**. Reversing the C–S linker between c-2 and the phenyl ring gave inactive **29**. Re-introducing the favourable 2,3-difluoro substitution gave analogue **30** but this was 70-fold less potent than the parent compound **20**. Turning our attention to the c-5 nitrile position, unsubstituted **31** was comparable in activity to **20** but weaker deactivating groups such as bromo **32** and iodo **33** led to a 10-fold drop in potency when compared to **31**.

In vitro and in vivo pharmacokinetic data was obtained for key compounds throughout this study and the results for compound **20**[19] are shown in Table 5. This compound has a reasonable lead like profile with acceptable potency in the binding and functional assays. The in vitro rat and human microsomal clearance is low to moderate and the compound is a weak acid (p*K*a 8.9) with moderate to high lipophilicity. Plasma protein binding as a consequence is high and the compound has a suitable rat in vivo profile with low clearance and good oral bioavailability. The PK

Table 5
Lead profile of pyrimidine-5-carbonitrile **20**



Measure	Triazolopyrimidine **20**
hrCXCR2 [^{125}I]-GRO-α IC_{50}	0.04 μM
[35S]-GTPγS IC50	0.14 μM
Rat microsome Cl (ml/min/kg)	45
Human microsome Cl (ml/min/kg)	14
Rat iv Cl (ml/min/kg)	1.6
Rat iv *Vss* (l/kg)	0.2
Rat iv *t½* (h)	6
Rat po bioavailability (%)	>100[a]
Rat plasma protein binding (%)	>99
Molecular weight	319
HT-eq Solubility pH 6.8 (g/l)	0.01
*c*Log*P*	3.9
Functional LLE (pIC50—Log*P*[b])	3.0

[a] Plasma concentrations in many samples were outside the range of the calibration curve—PK parameters should therefore be viewed as approximations.
[b] Compound **20** is weakly acidic and for the purposes of this calculation one can assume Log*P* = Log*D*.

profile suggests that clearance via glucuronidation is not an issue. Metabolite identification studies indicated the formation of sulfone and sulfoxide metabolites in vitro and in vivo but further studies are required to determine the levels of each.[20] LLE is improved significantly relative to the HTS hit **4** and compound **20** was inactive (IC_{50} >10μM) against a broader panel of 49 GPCRs. Pyrimidines exemplified by compound **20** formed the basis for on-going studies with this series of compounds.

In conclusion, HTS identified novel pyrimidine-5-carbonitrile chemotypes as selective CXCR2 antagonists. The SAR of the scaffold was explored, resulting in identification of pyrimidine-5-carbonitrile-6-cyclopropyl **20** which is a functional antagonist of the human CXCR2 receptor and shows good oral bioavailability in the rat.

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13. For full details of the biological assays used see: Porter, D.; Press, N. J.; Spanka, C. U.S. Pat. Appl. US 20100069407, 2010; (i) receptor binding assay: [^{125}I]-IL-8 hrCXCR2 was performed in a 96-well micro plate format with each reaction mixture containing 0.05 mg/ml CXCR2 membrane protein. Compounds of interest were pre-dissolved in DMSO so as to reach a final concentration of between 10 μM and 0.0005 μM [final concentration of DMSO 2% (v/v)]. Binding was initiated by addition of 0.02 nM [^{125}I]-IL-8; (ii) [^{35}S]-GTPγS binding assay for hrCXCR2 receptor using SPA technology: assay was performed in duplicate in 96 well Optiplate™ microplates in a final volume of 250 μl per well. Compounds were diluted in DMSO (0.5% final concentration). Data were expressed as the% response to 100 nM IL-8 minus basal.

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19. *Selected data for* ***20****:* δ_H (500 MHz, DMSO-d_6) 13.59 (1H, br), 7.38 (1H, m), 7.27 (1H, m), 7.23 (1H, m), 4.46 (2H, s), 2.15 (1H, m), 1.17 (2H, m), 1.07 (2H, m); $C_{15}H_{11}F_2N_3OS$ requires C, 56.42; H, 3.47; N, 13.16; S, 10.04. Found: C, 55.52; H, 3.46; N, 12.88; S, 9.56.
20. Glutathione conjugates were detected in vitro following incubation with reduced glutathione (GSH) in rat and human liver microsomes. Incubation was carried out for 60 min with 0.1 mg/ml microsomal protein and 5 mM GSH +/− oxidation co-factor NADPH. Samples were analysed by full scan LC–MS/MS following precipitation with MeCN and detection was confirmed by accurate mass measurement.