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Discovery and evaluation of a novel monocyclic series of CXCR2 antagonists

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ABSTRACT

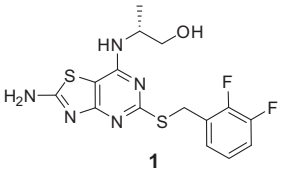
Antagonism of the chemokine receptor CXCR2 has been proposed as a strategy for the treatment of inflammatory diseases such as arthritis, chronic obstructive pulmonary disease and asthma. Earlier series of bicyclic CXCR2 antagonists discovered at AstraZeneca were shown to have low solubility and poor oral bioavailability. In this Letter we describe the design, synthesis and characterisation of a new series of monocyclic CXCR2 antagonists with improved solubility and good pharmacokinetic profiles.

Chemokines are a large family of structurally related small proteins which play an important role in the recruitment and activation of inflammatory cells. They are classified according to the nature of their conserved N-terminal cysteine residues, with the majority of human chemokines belonging to the CXC or CC groups. Chemokine receptors are class A G-protein coupled receptors which are usually activated by one or more chemokines from the same group.

The chemokines CXCL8 (interleukin-8, IL-8) and CXCL1 (growth-related oncogene α, GROα) play a role in the activation and recruitment of neutrophils to sites of inflammation mediated through the CXCR2 receptor.[1,2] Elevated levels of CXCL8 have been observed in several inflammatory diseases in man such as arthritis,[3] chronic obstructive pulmonary disease (COPD),[4] asthma[4] and psoriasis.[5] Additionally, studies performed with CXCR2 knockout mice show elevated lymphocytes without apparent pathogenic consequences, indicating that the effect of the CXCR2 receptor on normal non-pathogenic physiology is limited.[6] Blockade of the CXCR2 receptor, therefore, represents an attractive strategy for the treatment of inflammatory disorders. A number of groups have published research on small molecule antagonists of the CXCR2 receptor[7] and several compounds have advanced to clinical trials.[8]

We have also published previously in this area, disclosing several series of bicyclic CXCR2 antagonists (Fig. 1).[9]

Oral bioavailability of the early AstraZeneca CXCR2 antagonists was limited by their low solubilities. This was attributed to their bicyclic core structures and the potential to form a lattice of intermolecular H-bonds in the solid state. Disruption of crystal packing can be used to improve solubility[10] so we adopted a strategy of designing new monocyclic core structures, retaining the key acidic motif thought to be valuable for CXCR2 receptor binding.[7b]



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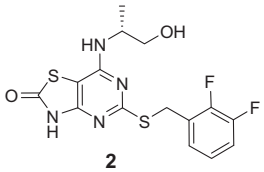

2

	1	2
CXCR2 binding pIC_{50}	8.4	9.0
Solubility (μM)	3.7	0.3
rat bioavailability, %	9	22

Figure 1. Previously disclosed AstraZeneca bicyclic CXCR2 antagonists.

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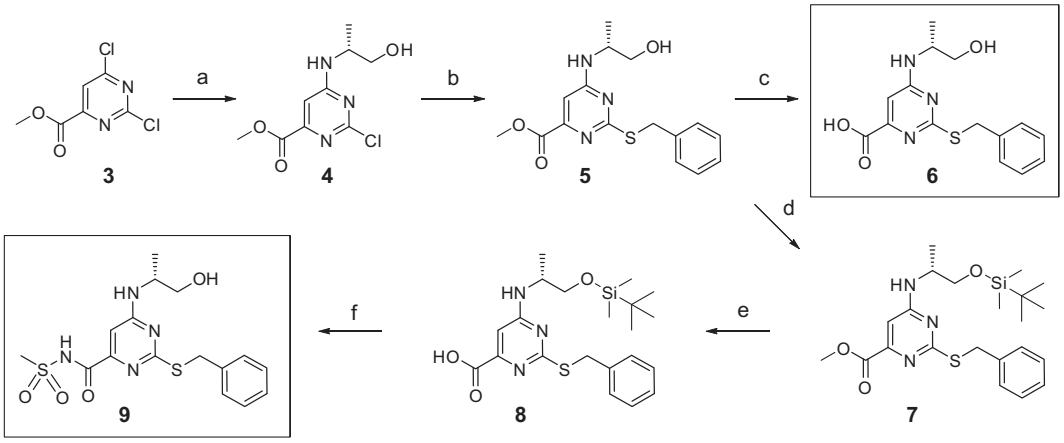
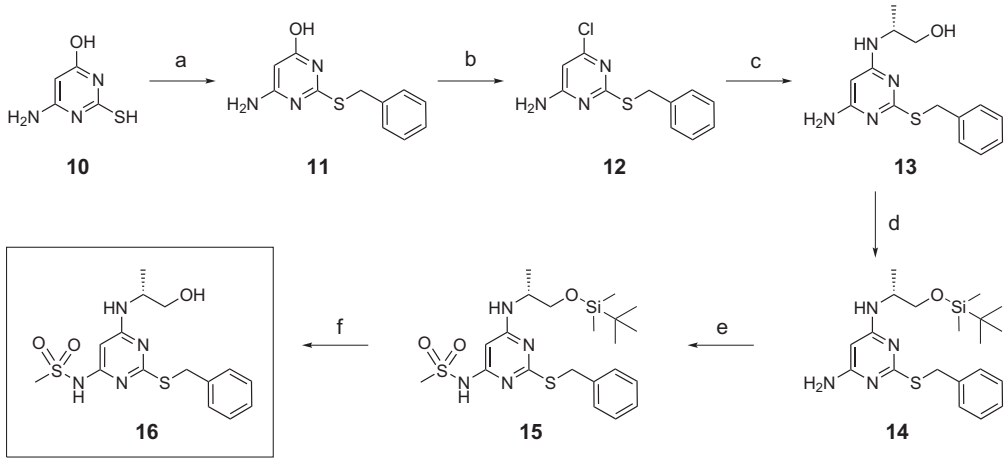
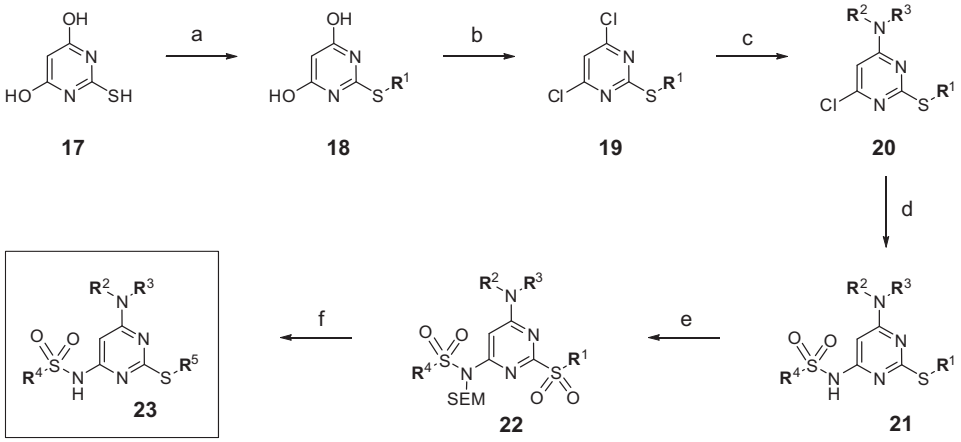


Scheme 1. Reagents and conditions: (a) (*R*)-alaninol, Hünig's base, THF, 74%; (b) benzyl mercaptan, NaH, THF/DMF, 10%; (c) 1 M aq LiOH, THF, 35%; (d) TBDMSCl, imidazole, DMF, ~100%; (e) 2 M aq NaOH, MeOH, 51%; (f) (i) $MeSO_2NH_2$, EDCI, DMF then (ii) 2 M aq HCl, MeOH, 18% over 2 steps.



Scheme 2. Reagents and conditions: (a) KOH, BnBr, THF/H_2O, 43%; (b) $POCl_3$, *N*,*N*-dimethylaniline, reflux, 75%; (c) (*R*)-alaninol, Hünig's base, NMP, 91%; (d) TBDMSCl, imidazole, DMF, 90%; (e) (i) $MeSO_2Cl$, Hünig's base, DCM then (ii) K_2CO_3, MeOH, ~100%; (f) TBAF, THF, 18%.



Scheme 3. Reagents and conditions: (a) R^1Br, NaOAc, MeCN/H_2O; (b) $POCl_3$, $Et_3BnN^+Cl^-$, DME, reflux; (c) NHR^2R^3, Na_2CO_3, MeCN, reflux; (d) $R^4SO_2NH_2$, $Pd_2(dba)_3$, XPhos or XantPhos, Cs_2CO_3, dioxane, 80 °C; (e) (i) SEMCl, Hünig's base, DCM then (ii) mCPBA (2 equiv), DCM; (f) (i) NaSH (5 equiv), R^5Br, DMSO then (ii) TFA.

This Letter describes the identification and evaluation of a series of monocyclic CXCR2 antagonists.[11]

Schemes 1 and 2 illustrate synthetic routes to initial monocyclic compounds; carboxylic acid **6**, acylsulfonamide **9** and methylsulfonamide **16**.

An alternative route to the sulfonamides is shown in Scheme 3. This route provided a more flexible approach to these compounds allowing diversification of the sulfide SR^1, amino group NR^2R^3 or sulfonamide $NHSO_2R^4$. The successful development of a palladium-catalysed synthesis of (hetero)aryl sulfonamides[12] allowed

access to sulfamides (R^4 = substituted amino) as well as sulfonamides (R^4 = alkyl, (hetero)aryl).

Initial results are shown in Table 1. Monocyclic analogues **6**, **9** and **16**, retaining key (*R*)-alaninol and *S*-benzyl units from our previous studies,[9] all showed a marked improvement in solubility compared to bicyclic compounds **1** and **2**, as well as promising activity against CXCR2. The benzoic acid **6** and its acyl sulfonamide analogue **9** have lower activity than the sulfonamide **16**, indicating that the acidic centre may not be optimally positioned for CXCR2 receptor binding. Additionally, the high plasma protein binding for **6** relative to log*D* was predicted to cause difficulties in achieving sufficiently high free blood concentrations. The pyrimidine sulfonamide **16** therefore became a new starting point for lead optimisation.

Optimisation of **16** began by investigation of the *S*-benzyl and 4-amino substituents (Table 2). SAR in the sulfonamide series was found to be parallel to that seen for the earlier bicyclic compounds,[9] and optimal potency was achieved with (*R*)-alaninol as the amine substituent, together with a 2,3-dihalobenzyl on the sulfur.

We then investigated the effect of varying the sulfonamide (Table 3). Replacement of methyl with higher alkyl analogues (compounds **31** and **32**) offered little advantage. Potency was not significantly improved and in the case of the most lipophilic benzyl analogue **32**, plasma protein binding was very high. The trifluoromethyl analogue **33** had pIC_{50} 8.0 which is probably due to its increased acidity.[7b] This is also true of the benzene sulfonamide analogues **34**–**36**, where the observed potencies against the CXCR2 receptor parallel the relative acidities of the compounds. Unfortunately, the more acidic analogues suffer from high plasma protein binding. An interesting observation is that primary sulfamide **37** is inactive at CXCR2. We postulate that this is due to intramolecular hydrogen bonding between the NH_2 and the pyrimidine N to form a six-membered ring,[18] and suggest that this is not an active conformation. Further evidence is provided by the positive activity data for a similar sulfamide **38** in which intramolecular H-bonding is not possible.

Encouraged by these results, we investigated the benzene sulfonamide and the sulfamide series further, choosing to use 2,3-difluorobenzyl and 2-fluoro-3-chlorobenzyl substituents on sulfur, which were optimal for potency (Table 4). Changes to the sulfonamide were well tolerated in terms of potency and solubility, and offered an opportunity to modulate the overall physicochemical properties of this series. Introduction of heteroatoms into the benzene sulfonamides provided more polar analogues with lower plasma protein binding, illustrated by imidazole analogues **39** and **40**. Unfortunately, these compounds showed a significant drop-off in potency when tested in a CXCR2 cell-based assay.[19] An explanation of this could be that the compounds do not penetrate the cell membrane effectively during the time frame of the in vitro assay, and that binding to the receptor is at an intracellular site as suggested by previously reported pharmacology.[20] More significant cell potency drop-off is seen with piperazine **42**. The addition of a basic centre, creating a zwitterionic compound, is tolerated in terms of receptor binding but reduced cell permeability results in a drop in cell potency. A range of other sulfamides (**43**–**48**) have excellent overall profiles with required potency in both binding and cellular assays, solubility and hPPB. From a metabolic stability viewpoint, azetidine (**43** and **44**) and morpholine (**47** and **48**) were more stable than pyrrolidine (**45**) and piperidine (**46**) analogues. Metabolite identification studies in human hepatocytes showed that these sulfamides have three major sites of oxidative metabolism: debenzylation of sulfur, N-dealkylation of the sulfamide, and oxidation of the (*R*)-alaninol primary alcohol. The increased stability of the azetidine and morpholine analogues is probably a composite of both lower lipophilicity and reduced potential for N-dealkylation.

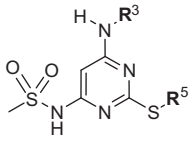
Key compounds were tested in in vivo pharmacokinetic studies in rat[23] and compared to earlier bicyclic compounds (Table 5 and Fig. 2). Whilst maintaining comparable binding and cell potency, the new pyrimidine sulfamide compounds showed large improvements in solubility over **1** and **2** and improved overall pharmacokinetic profiles. Furthermore, compounds **43** and **47** have lower intrinsic clearances in human hepatocytes, offering the potential for in vivo profiling of this series in animal models of inflammatory disease and the possibility of profiling in man.

In conclusion, we have disclosed a novel monocyclic series of pyrimidine sulfamide CXCR2 receptor antagonists. These are potent and soluble compounds with favourable pharmacokinetic and physicochemical properties. Key findings from this programme of research showed that breaking the bicyclic core of our previously disclosed CXCR2 antagonists was an effective way of significantly improving solubility. These compounds represent

Table 1
Monocycles versus bicycles: CXCR2 binding potency,[13] solubility,[14] pK_a,[15] human plasma protein binding (hPPB)[16] and log*D*[17] for **1**, **2**, **6**, **9**, **16**

Compound	CXCR2 pIC_{50}	Solubility (μM)	pK_a	hPPB, % free	log*D*
1	8.4	3.7	—	2.6	3.3
2	9.0	0.3	6.7	0.3	3.2
6	6.4	150	5.5	1.0	−0.7
9	6.1	>120	4.9	7.0	−0.7
16	7.1	>100	6.9	1.9	1.9

Table 2
Investigation of methane sulfonamide SAR: CXCR2 potency[13] and solubility[14] for **16**, **24**–**30**



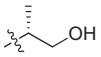
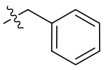
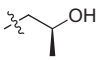
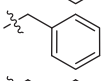
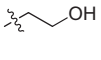
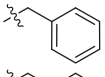
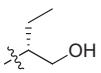
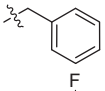
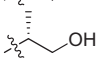
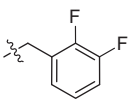
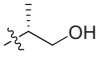
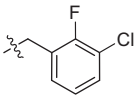
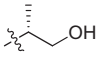
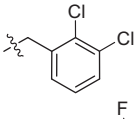
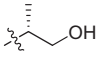
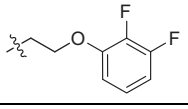
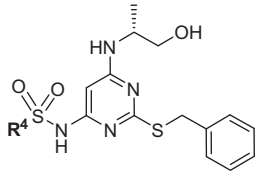
Compound	R^3	R^5	CXCR2 pIC_{50}	Solubility (μM)
16			7.1	>100
24			5.1	>130
25			5.5	59
26			6.4	—
27			7.5	30
28			7.9	21
29			6.8	100
30			6.2	94

Table 3
Investigation of sulfonamide substituent SAR: CXCR2 potency,[13] solubility,[14] pK_a,[15] human plasma protein binding (hPPB)[16] and log$D$[17] for compounds **16**, **31**–**38**



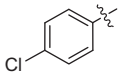
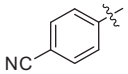
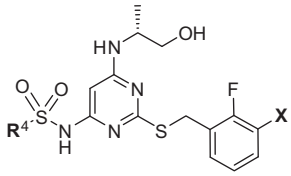
Compound	R^4	CXCR2 pIC_{50}	Solubility (μM)	pK_a	hPPB, % free	log$D$
16	Me	7.1	>100	6.9	1.9	1.9
31	*n*Pr	6.8	120	–	–	–
32	Bn	7.3	100	–	<0.1	2.7
33	CF_3	8.0	>90	5.8	<0.1	2.2
34	Ph	7.1	110	6.6	<0.2	2.5
35		7.5	82	6.5	<0.1	3.5
36		7.6	>110	6.1	<0.1	2.6
37	NH_2	<5.0	–	–	–	–
38	NMe_2	7.2	–	7.3	1.9	2.6

Table 4
Further investigation of sulfonamide substituent SAR: CXCR2 potency,[13] CXCR2 cell potency,[19] solubility,[14] human plasma protein binding (hPPB),[16] human hepatocyte intrinsic clearance (HH Cl_{int}),[21] log$D$[17] and Caco-2[22] for compounds **39**–**48**



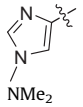
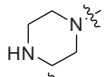
	R^4	X	CXCR2 pIC_{50}	CXCR2 cell pA_2	Solubility (μM)	hPPB, % free	HH Cl_{int} (μL/min/10^6 cells)	log$D$	Caco-2 A to B P_{app} (10^{-6} cm/s)
39		F	7.5	–	100	2.1	–	1.6	–
40		Cl	7.9	6.7	53	1.9	–	2.0	1.4
41	NMe_2	Cl	8.3	–	–	–	–	3.4	6.4
42		Cl	8.1	<6.5	77	3.7	–	1.5	0.1
43		F	8.4	8.7	69	1.0	3.1	2.6	10.1
44		Cl	8.6	8.0	56	<0.2	2.4	3.2	–
45		F	8.1	7.9	>110	0.6	12	3.4	7.5
46		F	8.1	7.8	100	1.4	30	3.9	–
47		F	8.4	8.0	62	3.0	4.6	2.4	7.0
48		Cl	8.3	7.8	73	1.5	8.0	3.1	5.4

Table 5
Overall profile comparison of earlier bicyclic compounds with new pyrimidine sulfonamides: CXCR2 potency,[13] CXCR2 cell potency,[19] solubility,[14] human hepatocyte intrinsic clearance (HH Cl_{int})[21] and rat in vivo pharmacokinetics (bioavailability (F and F_{abs}, calculated using a rat liver blood flow of 70 mL/min/kg), clearance (Cl), steady state volume (V_{ss}), half life after IV dosing ($t½$) and extrapolated oral area under the curve normalised to 1 μmol/kg (AUC)) for compounds **1**, **2**, **43** and **47**[24]

Compound	CXCR2 pIC_{50}	CXCR2 cell pA_2	Solubility (μM)	HH Cl_{int} (μL/min/10^6 cells)	F (%)	F_{abs} (%)	Cl (mL/min/kg)	V_{ss} (L/kg)	t½ (h)	AUC (μM.h)
1	8.4	7.9	3.7	7.6	9	12	17	1.0	1.2	0.7
2	9.0	8.9	0.3	15	22	24	6	0.3	1.1	2.1
43	8.4	8.7	69	3.1	44	50	8	1.4	3.2	5.9
47	8.4	8.0	62	4.6	31	48	25	1.1	1.0	1.6

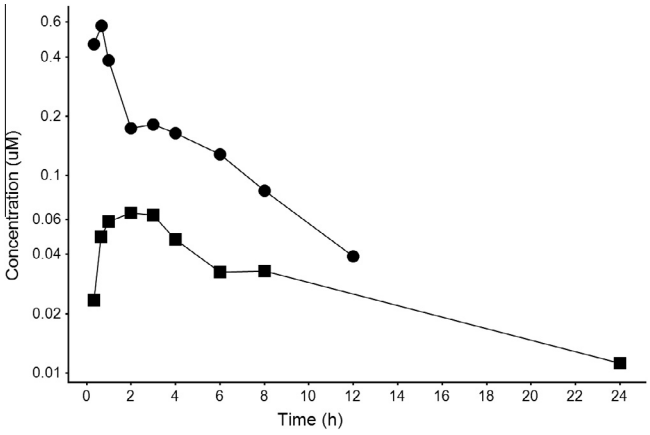


Figure 2. Oral pharmacokinetic profiles for compounds **2** (squares) and **43** (circles) in rat normalised to 1 μmol/kg.

excellent investigational tools for in vivo models of inflammatory diseases and the results of this work will be the subject of future publications.

References and notes

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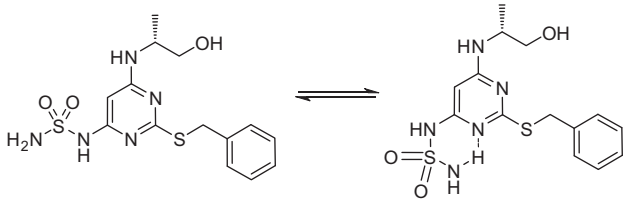
14. 8 μL of a 5 mg/mL solution of compound in DMSO was diluted in 792 μL 0.1 M pH 7.4 phosphate buffer. The solution was shaken overnight then filtered, analysed by HPLC and the solubility calculated by UV quantification using comparison with a 0.05 mg/mL solution in DMSO. Solubilities were determined on free acids.
15. pK_a values were determined from changes in UV spectra as a function of pH using a GLpKa auto titrator with DPAS attachment (Sirius Analytical Instruments Ltd, Forest Row, UK).
16. Human plasma protein binding was determined by equilibrium dialysis of the compound between plasma and phosphate buffered saline at 37 °C, followed by HPLC analysis with UV or MS detection.
17. Log*D* was measured using a shake flask technique followed by HPLC analysis with quantitative MS.
18. 
19. CXCR2 cell activity was determined using HEK cells transfected with the CXCR2 receptor. Mobilisation of intracellular calcium was measured using the FLIPR system. For full details see Ref. 20a. IC_{50} values are the mean of at least two independent observations. Errors are within ±20%.
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23. All animal studies were carried out under licences issued under the Animals (Scientific Procedures) Act 1986 following local ethical committee review.
24. All compounds for IV dosing were formulated in DMA/PEG/saline. All compounds for oral dosing were formulated in Tween/CMC except for **47**, which was formulated in DMA/PEG/saline.