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Evaluation of a series of bicyclic CXCR2 antagonists

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Abstract—The CXCR2 SAR of a series of bicyclic antagonists such as the 2-aminothiazolo[4,5-*d*]pyrimidine **3b** was investigated by systematic variation of the fused pyrimidine-based heterocyclic cores. Replacement of the aminothiazole ring with a 2-thiazolone alternative led to a series of thiazolo[4,5-*d*]pyrimidine-2(3*H*)-one antagonists with markedly improved biological and pharmacokinetic properties, which are suitable pharmacological tools to probe the in vivo effects of CXCR2 antagonism combined with the associated CCR2 activity.

Chemokines are a set of small proteins typically comprising 70–80 amino acids which play an important role in the recruitment and activation of inflammatory cells. They may be classified according to the nature of conserved cysteine motifs, and generally fall into two main categories, the CC and CXC chemokines. Both subfamilies include a number of potent chemoattractants and activators of different leukocyte subsets. For example, the CXC chemokines interleukin-8 (IL-8) and growth-related oncogene-α (GRO-α) modulate the activity of neutrophils, whereas the CC chemokine monocyte chemoattractant protein-1 (MCP-1) influences the behaviour of monocytes and T lymphocytes. Upregulation of these chemokines has been associated with several inflammatory diseases in man.[1,2] In such diseases leukocytes are directed to the site of infection or injury by a chemical gradient of chemokines, a process which is mediated by interaction with G-protein coupled receptors on the leukocyte surface. Blockade of these chemokine receptors represents an attractive strategy for therapeutic intervention in inflammatory diseases, provided the key chemokine–receptor interactions can be targeted. The CXCR2 receptor, found on neutrophils, binds a number of neutrophil activating chemokines including IL-8 with high affinity.[3] The CCR2 receptor, found on monocytes, likewise interacts with several CC ligands such as MCP-1.[4] Both of these pathways independently present tempting approaches for small molecule antagonism; the development of an antagonist with activity at both receptors would offer a particularly novel opportunity for disease modification, since no such dual antagonists have been reported to date.

We recently disclosed a series of potent 2-aminothiazolo[4,5-*d*]pyrimidine CXCR2 antagonists such as **1** (Fig. 1) derived from a hit-to-lead study on the HTS hit **2**.[5] Subsequent exploration of the 5-thio- and 7-amino-substituent SAR resulted in the optimised aminothiazole antagonist **3b**,[6] which possessed improved potency but still suffered from relatively poor rat bioavailability

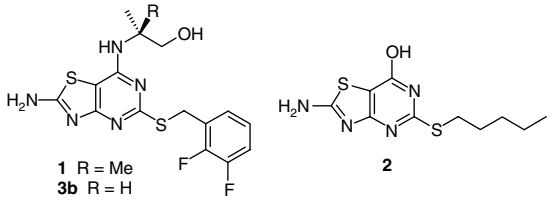

Figure 1.

Keywords: CXCR2; Thiazolo[4,5-*d*]pyrimidine-2(3*H*)-one.
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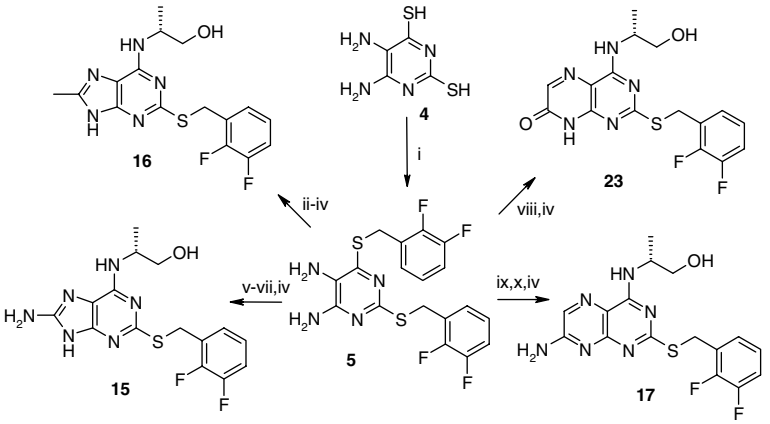
(Table 1). In order to address this issue, a programme of structural variation of the central bicyclic heterocycle was undertaken, the results of which are presented in this paper.

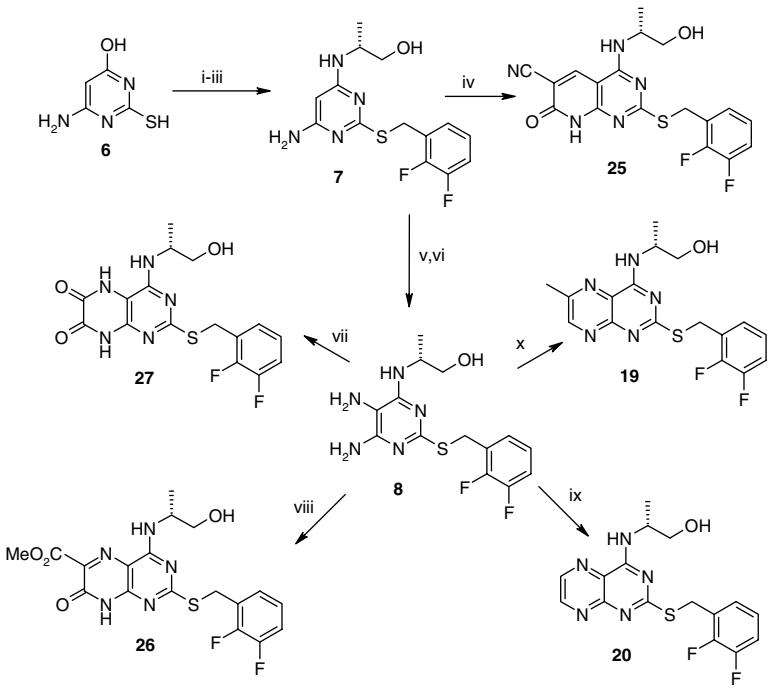
The synthetic routes to the chemokine antagonists described in this paper are depicted in Schemes 1–4. Although substituent variation could be readily carried out by parallel synthetic chemistry methodology, changes to the heterocyclic core required a more individual approach. However, two synthetic pathways proved to be particularly useful for the construction of a range of different fused heterocyclic systems, the first of which, shown in Scheme 1, offered rapid access to several pteridine and purine templates. Alkylation of the commercially available bis-thio pyrimidine **4** afforded the common dibenzyl intermediate **5**, which could be converted into several different bicyclic systems by condensation with a range of electrophiles.[7] In each case, the peri-thiobenzyl substituent could be selectively displaced with a primary amine such as (*R*)-alaninol to produce the desired target. A second general route hinged on incorporation of a suitable amino alcohol substituent earlier in the synthetic process, as shown in Scheme 2, which allowed the construction of an alternative set of heterocyclic ring systems. Thus, alkylation of the mono-thio pyrimidine **6** followed by chlorination and displacement with (*R*)-alaninol gave the diamino pyrimidine intermediate **7**. This could be annulated with a cyanopyridone ring directly to give **25**, or converted via nitrosation and reduction[8] to the useful triamino pyrimidine intermediate **8** which was used to construct a range of different pteridine-based analogues.[9] The synthesis of heterocycles inaccessible by either of these two general routes is outlined in Schemes 3 and 4. Thus, the amino-pteridinone **9**[10] was diazotised giving the pteridine-dione **10** as a by-product. Bis-chlorination and alaninol displacement were followed by a Suzuki coupling with methylboronic acid to afford the 7-methyl-pteridine **18**. The 'reverse' aminothiazole **21** was derived from the dichloronitropyrimidine intermediate **11**[11] by sequential chloro-dis-

Table 1. CXCR2 potency,[14] rat oral bioavailability (*F*), rat in vivo clearance (Cl), human plasma protein binding (hPPB), pK_a[15] and log *D* for compounds **3b**, **15**–**29**, **30b**

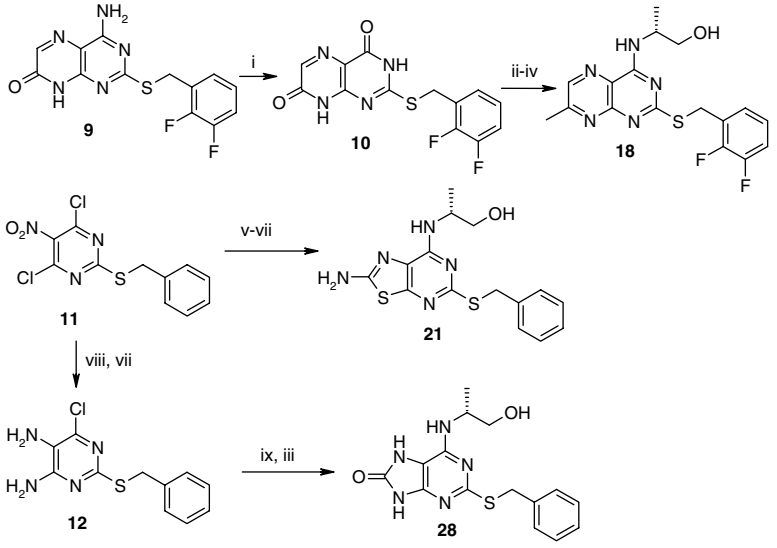
Compound	CXCR2 IC_{50}, nM	*F*, %	Cl, ml/min/kg	hPPB, %free	pK_a	log *D*
3b	4	9	17	2.6		3.3
15	13	5	31	2.3		3.2
16	630					
17	7	6	24	5.2		2.9
18	280					
19	140					
20	280					
21	850					
22	5	<5	44	1.1	4.7	0.9
23	1	44	21	1.2	7.5	3.1
24	350				10.5	3.4
25	16			1.7	7.9	3.3
26	2				6.6	2.5
27	45				6.5	2.5
28	2500				9.1	2.6
29	220				11.4	3.1
30b	1	22	6	0.29	6.7	3.2



Scheme 1. Reagents: (i) KOH, 2,3-difluorobenzyl bromide, MeOH (47%); (ii) Ac_2O; (iii) $SOCl_2$ (44%); (iv) (*R*)-alaninol, Hunig's base, NMP (40–50%); (v) ethoxycarbonyl isothiocyanate, MeCN; (vi) diisopropylcarbodiimide (73%); (vii) LiOH, THF, water (65%); (viii) ethyl glyoxalate, Na, MeOH (46%); (ix) $BrCH_2CN$, Hunig's base, DMSO (29%); (x) KOH, MeOH (26%).

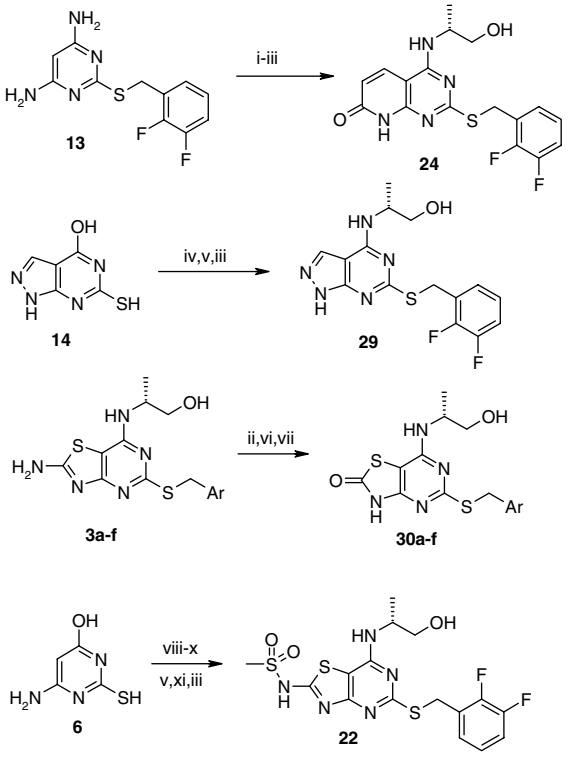


Scheme 2. Reagents: (i) KOH, 2,3-difluorobenzyl bromide, aq DMF (89%); (ii) $POCl_3$, 2-picoline (44%); (iii) (*R*)-alaninol, Hunig's base, NMP (90%); (iv) H_2CO, ethyl cyanoacetate, Et_3N, EtOH (17%); (v) $NaNO_2$, aq AcOH (92%); (vi) $Na_2S_2O_4$, aq DMF (69%); (vii) diethyl oxalate (13%); (viii) diethyl ketomalonate, Na, MeOH (27%); (ix) glyoxal, NMP (13%); (x) 1,3-dihydroxyacetone, NaOAc, MeOH (47%).



Scheme 3. Reagents: (i) isopentylnitrite, bromoform, DMSO (30%); (ii) $POCl_3$ (68%); (iii) (*R*)-alaninol, Hunig's base, NMP (40–50%); (iv) $CH_3B(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, dioxane (10%); (v) (*R*)-alaninol, Hunig's base, THF (64%); (vi) KSCN, DMF (95%); (vii) iron, NH_4Cl, aq EtOH (80–90%); (viii) NH_3, Hunig's base, dioxane (52%); (ix) triphosgene, Et_3N, DCM (70%).

placement with alaninol and thiocyanate followed by nitro reduction and spontaneous cyclisation. The same intermediate **11** also gave rise to the imidazolidinone **28** via displacement with ammonia and nitro reduction to give **12** followed by cyclisation with triphosgene and alaninol incorporation. The pyridone **24** was made from the diaminopyrimidine **13**[10] by condensation with ethyl 3-ethoxyacrylate[12] followed by diazotisation/bromination and alaninol displacement (Scheme 4). The pyrazolopyrimidine **29** was readily formed from the commercially available precursor **14** by alkylation, chlorination and alaninol displacement. Conversion of the aminothiazole antagonists **3a**–**f** into the corresponding thiazolones **30a**–**f** was simply effected by diazotisation of the 2-amino group to the bromide, displacement with methoxide and acidic hydrolysis. Finally, synthesis of the methylsulfonate derivative **22** of **3b** started with the mono-thio pyrimidine **6** which was treated to a sequence of alkylation, thiocyanation and ring closure steps.[13] Subsequent chlorination, sulfonation and reaction with (*R*)-alaninol gave the sulfonamide **22**.



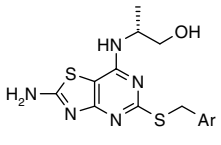
Scheme 4. Reagents: (i) ethyl 3-ethoxyacrylate, AcOH (61%); (ii) isopentylnitrite, bromoform, DMSO (25–50%); (iii) (*R*)-alaninol, Hunig's base, NMP (50–70%); (iv) NaH, 2,3-difluorobenzyl bromide, DMF (51%); (v) $POCl_3$, $PhNMe_2$ (50–80%); (vi) KOH, MeOH (79%); (vii) aq HCl, dioxane (54%); (viii) KOH, 2,3-difluorobenzyl bromide, DMF, water (89%); (ix) KSCN, Br_2, pyridine, DMF (95%); (x) DMF, water (88%); (xi) $MeSO_2Cl$, Hunig's base, THF (50%).

The set of bicyclic CXCR2 antagonists described herein may be subdivided into two broad categories of heterocycle according to the presence or absence of an acidic hydrogen atom. The utility of this distinction is borne out by the different physicochemical and pharmacokinetic properties of the two subclasses of compounds, most notably the poor rat bioavailability of the neutral series and the high plasma protein binding of the acidic heterocycles, and so the SAR of the two types of antagonist will be outlined in turn. It should be noted that these compounds were designed as part of an investigation specifically targeting CXCR2 antagonism. Subsequent selectivity profiling of particular antagonists revealed a correspondingly high CCR2 activity. However, these compounds were not optimised against CCR2 and so the focus of this discussion will be SAR at the CXCR2 receptor.

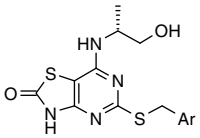
Previous studies on the series of 2-aminothiazolopyrimidines such as **1**[5,6] had determined the optimal combination of substituents attached to the central heterocyclic ring system. A 5-thiobenzyl substituent was necessary for good potency, with 2,3-difluoro substitution proving optimal, and a 7-amino-alcohol group was required, with (*R*)-alaninol the most potent. To facilitate fair comparison, these two substituents were incorporated in the majority of compounds prepared for this study. The tacit assumption that the substituent SAR is parallel across different heterocyclic systems was demonstrated explicitly in the one case of the thiazolo[4,5-*d*]pyrimidine-2(3*H*)-one antagonists described below (Table 2). The prior survey of thiazolopyrimidine SAR[5,6] had also highlighted the importance of the primary 2-amino substituent for potency against CXCR2. This principal activity requirement was found to be shared by other potent neutral antagonists in this series (Table 1). Thus, simply exchanging the thiazolo sulfur atom of **3b** for a nitrogen to generate the corresponding 8-aminopurine **15** maintains activity against CXCR2. However, replacing the amino group of **15** with a methyl substituent afforded the markedly less active compound **16**. Similarly, changing the amino group of the potent pteridine antagonist **17** to a methyl group at either of the two free positions to give **18** and **19** results in a pair of compounds with greatly reduced potency, which is also the case for the unsubstituted pteridine **20**. The presence of an amino group is no guarantee of potency, however, as can be seen with the 'reversed' 2-aminothiazolo[5,4-*d*]pyrimidine **21**. This compound is significantly less potent than its [4,5-*d*] isomer **3d** (Table 2), suggesting a preference for a particular spatial orientation of the hydrogen bonding elements of the aminothiazole ring. Although the amino group of the neutral antagonists such as **3b**, **15** and **17** may have a beneficial influence on their CXCR2 antagonist potencies, it does not appear to be conducive to achieving good rat oral bioavailabilities (Table 1). These three compounds all have bioavailabilities below 10% despite possessing physicochemical properties (such as log *D*) usually concomitant with good absorption, and in vivo clearances low enough to limit the impact of first pass metabolism. As a consequence, an effort was made to find potent CXCR2 antagonists within this series which lacked this primary amino group, in the hope of obtaining an improved in vivo PK profile. An attractive approach entailed substitution of the primary amino group to reduce the number of H-bond donors, in case this factor was absorption-limiting. Although alkylation of the amino group was known to reduce potency,[6] it was found that the sulfonamide derivative **22** retained all its activity. While no improvement in rat bioavailability was seen with this compound, perhaps due to its much lower log *D* and/or higher clearance, its potency encouraged an extensive survey of acidic heterocyclic ring systems in order to explore the relationships between pK_a, potency and bioavailability (Tables 1 and 2).

The marked influence of pK_a on potency can be clearly seen in the 6,6-fused heterocyclic compounds **23**–**27**. Thus, the least acidic example prepared, the pyridone **24**, was a relatively weak antagonist. Lowering the pK_a by adding a 6-cyano substituent to give **25** brought about a significant improvement in potency, a change that was all the more striking in the two more acidic pteridinone analogues **23** and **26**, which achieved nanomolar potencies. Gratifyingly, the pteridinone **23** also possessed good rat bioavailability, in contrast to all of the neutral congeners tested. pK_a is not the only relevant factor in determining the activity of this series, however, as demonstrated by the drop in potency of the acidic pteridinedione antagonist **27**. The 5,6-fused heterocyclic

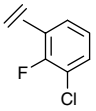
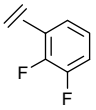
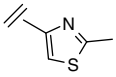
Table 2. CXCR2 potency,[14] rat oral bioavailability (*F*), rat in vivo clearance (Cl), human plasma protein binding (hPPB) and CCR2 potency[16] for compounds **3a–f**, **30a–f**



3a-f



30a-f

Compound	Ar	CXCR2 IC_{50}, nM	*F*, %	Cl, ml/min/kg	hPPB, %free	CCR2 pA_2, nM
3a		3				
30a		1	81	4	0.08	8
3b		4	9	17	2.6	16
30b		1	22	6	0.29	6
3c		13		25		
30c		2	39	10	0.16	
3d		13	5	32	5.5	
30d		5	56	7	0.27	
3e		35			12	
30e		5	41	17	1.8	
3f		120	<1	43		
30f		60	17	6	0.87	

analogues **28–30** also appear to show some dependence on pK_a. The essentially neutral pyrazole **29** has a similar CXCR2 potency to the comparable pyridone **24**. Increasing the acidity a hundredfold in the imidazolidinone **28** was accompanied by a drop in activity, but this is presumably due in part to the non-optimal 5-thiobenzyl substituent. However, lowering the pK_a further by replacing one of the NH moieties of the imidazolidinone with a sulfur atom gave the most potent class of CXCR2 antagonists, the thiazolo[4,5-*d*]pyrimidine-2(3*H*)-ones **30a–f** (Table 2). Thus, the direct analogue of **28**, the thiazolone **30d** (pK_a = 7.0), is over a hundred times more potent, and incorporation of optimal 5-substituents in **30a** and **30b** once more afforded low nanomolar CXCR2 antagonists with acceptable rat bioavailabilities. The thiazolo[4,5-*d*]pyrimidine-2(3*H*)-one-based antagonists were found to possess the best combination of potency and pharmacokinetic properties, and several analogues were prepared bearing different substituents. Some of the properties of these compounds are tabulated in Table 2 and compared with the corresponding 2-aminothiazolo[4,5-*d*]pyrimidines. The broadly parallel nature of the substituent SAR of these two series is readily apparent, perhaps an unexpected property given the presumably different binding nature of the neutral aminothiazole and acidic 2-thiazolone key pharmacophores. Also noteworthy is the fact that representative compounds from these series show comparable activity at the CCR2 receptor (Table 2). The higher intrinsic potency of the thiazolo[4,5-*d*]pyrimidine-2(3*H*)-ones is partially offset by their higher plasma protein binding. However, this is more than compensated for by their higher rat bioavailabilities and lower metabolic clearances, offering the potential for detailed in vivo profiling of this series in animal models of inflammatory disorders.

In conclusion, this survey of the heterocyclic core of a series of bicyclic CXCR2 antagonists has resulted in the discovery of a series of potent, orally bioavailable compounds based on a thiazolo[4,5-*d*]pyrimidine-2(3*H*)-one core, which additionally possess high affinity for the CCR2 receptor. The favourable pharmacokinetic and physicochemical properties of this series render them excellent investigative tools for the in vivo assessment of combined CXCR2 and CCR2 antagonism. The results of such studies will form the basis of forthcoming publications.

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14. CXCR2 binding affinity was determined via a scintillation proximity assay (SPA) using [^{125}I]IL-8 and human recombinant CXCR2 (hrCXCR2) receptor expressed in HEK 293 membranes. For full details, see: Austin, R.; Baxter, A.; Bonnert, R.; Hunt, F.; Kinchin, E.; Willis, P. Int. Patent Appl. WO 00/09511. IC_{50} values are means of at least two independent observations. Errors are within ±20%.
15. pK_a's 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. CCR2 potency was determined as follows: THP-1 cells were grown in RPMI medium containing 10% (v/v) FCS and 2 mM L-glutamine in a humidified incubator at 37 °C, 5% CO_2. THP-1 cells were harvested by centrifugation at 300*g* for 5 min at room temperature. The cells were resuspended in Tyrode's Buffer (10 mM Hepes, pH 7.4, 137 mM NaCl, 2.7 mM KCl, 0.4 mM sodium dihydrogen phosphate, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$) containing 5 μM Fluo3-AM and incubated at room temperature for 60 min. The Fluo-3AM solution was removed by centrifugation at 300*g* for 5 min at room temperature and the cell pellet resuspended in Tyrode's Buffer. The assay was performed in 96-well poly-D-lysine coated FLIPR plates. Compounds in 0.75% DMSO were incubated with 200,000 cells per well for 30 min at room temperature. Calcium transients in response to various concentrations of MCP-1 were then measured using a FLIPR™ (Molecular Devices) at room temperature, and pA_2 values for antagonism determined.