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SAR studies on thiazolo[4,5-*d*]pyrimidine based CXCR2 antagonists involving a novel tandem displacement reaction

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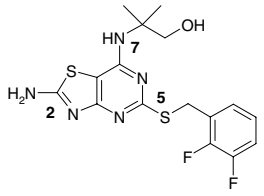
Abstract—As part of a Lead Optimisation programme to identify small molecule antagonists of the human CXCR2 receptor, a series of substituted thiazolo[4,5-*d*]pyrimidines was prepared via the application of a novel tandem displacement reaction.

Chemotatic cytokines (Chemokines) are a class of potent inflammatory mediators. Interaction of chemokines with specific cell populations is mediated by G-protein coupled receptors. These receptors are divided into four subtypes (CC, C, CX_3C and CXC), depending upon the position of the N-terminal cysteine residues within the protein. Interleukin 8 (IL-8, CXCL8) and growth-related oncogene α (GROα) are members of the CXC chemokine subfamily and 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 diseases such as arthritis,[3] chronic obstructive pulmonary disease (COPD), asthma,[4] ulcerative colitis and psoriasis.[5] In the light of this finding small molecule antagonists of the CXCR2 receptor are attractive biological targets.

As part of a Hit-to-Lead study, our laboratory has previously disclosed the identification of a series of novel thiazolopyrimidines as potent CXCR2 receptor antagonists.[6] Early evaluation had indicated that *S*-benzyl substitution at the 5-position and hydroxy-alkylamino substitution at the 7-position are preferred, exemplified by compound **1**, Table 1, which formed the basis of a new Lead Optimisation (LO) project. This paper will disclose a further programme of synthetic work towards primarily increasing potency and highlighting further structure activity relationship (SAR) within this series.

Simple variation of the 7-substituent of **1** used the same methodology applied during the Lead Identification (LI)

Table 1. Lead profile of thiazolopyrimidine **1**



Assay	Thiazolopyrimidine **1**
Binding CXCR2 pIC_{50}	7.6
Functional pIC_{50}	7.8[a]
Human plasma protein binding %	98.4
Solubility (pH 7.4) μg/ml	2.5[b]
Rat iv Cl ml/min/Kg	25
Rat iv V_{ss} 0.5 L/Kg	1.9
Rat iv $T_{1/2}$ h	1.2
Hu CaCo2 cm/s × 10^6	3.6
Rat po bioavailability %	15

[a] Functional antagonism was shown by blockade of GROα stimulated intracellular calcium mobilisation in isolated human neutrophils using a fluorescence imaging plate reader (FLIPR).[7]
[b] Solubility corrected from value given in reference 1, Table 5.

Keywords: CXCR2; Thiazolopyrimidine.
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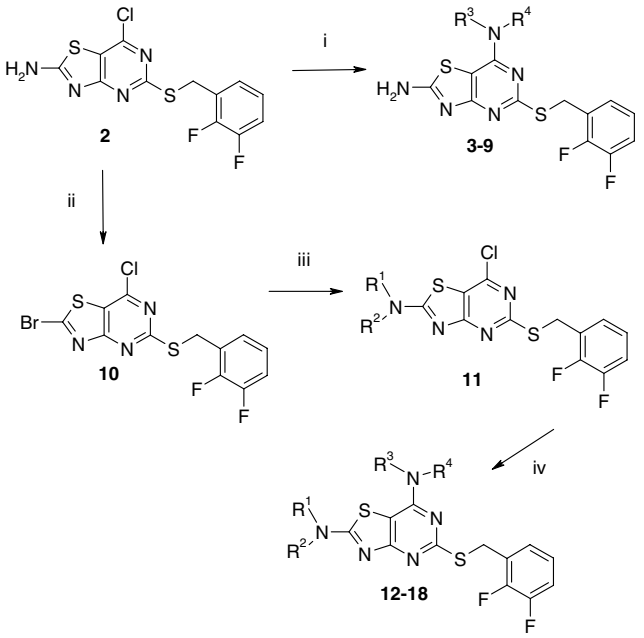
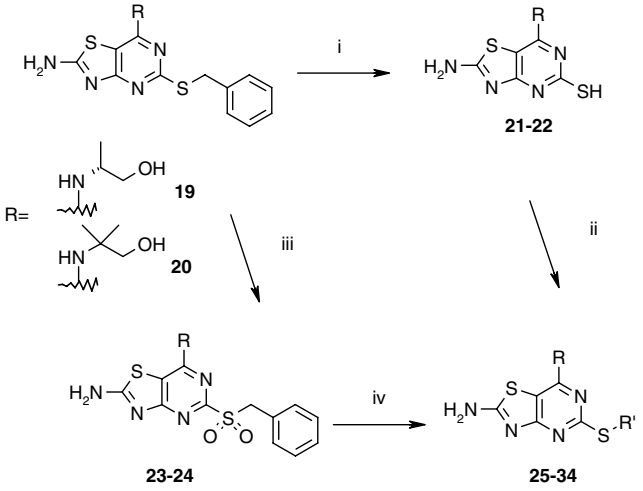


Scheme 1. Reagents and conditions: (i) R^3R^4NH 100 °C; (ii) $CHBr_3$, *iso*-amylnitrite, CH_3CN, 50 °C (82%); (iii) R^1R^2NH (rt), Et_3N; (iv) R^3R^4NH 100 °C.



Scheme 2. Reagents and conditions: (i) Na, NH_3,NH_4Cl (70%); (ii) R′Br, NaH (45–85%); (iii) Oxone™ (96%); (iv) R′SH, $NaBH_4$, DMSO, 60 °C (20–65%).

phase, which involved treatment of the chloro compound **2** with a range of primary and secondary amines at elevated temperatures[8] to give compounds **3**–**9**, Scheme 1. The development of a new tandem displacement reaction permitted the independent exploration of the SAR at both the 2- and 7-positions via a ''one pot'' procedure. This route exploits the difference in reactivity of positions 2 and 7 in compound **10** towards nucleophiles.

Diazotisation of **2** with *iso*-amyl nitrite in the presence of bromoform at 50 °C[9] afforded **10**. This intermediate was treated with an array of amines at room temperature affording the mono-substituted intermediates **11** efficiently. After an hour, a second set of amines was added and the temperature increased to 100 °C to give the disubstituted compounds **12**–**18**. Purification of the final compounds was performed using automated high performance liquid chromatography. The isolated products were quantified using Chemi-luminescent Nitrogen Detection (CLND)[10] and the purified samples were solubilised to a pre-determined concentration for in vitro biological screening (affinity data and in vitro DMPK data obtained). The biological activity of key compounds was confirmed using re-synthesised solid, fully characterised samples.

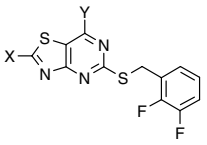
Two synthetic approaches were employed to explore the SAR at the 5-position. Both routes started with the benzylthio ethers **19**–**20**, Scheme 2. This strategy allowed the opportunity to increase the diversity of substitution of the products **25**–**34** by not limiting the synthesis to a single reagent class.

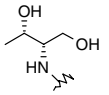
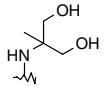
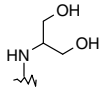
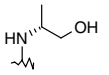
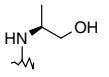
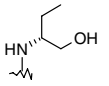
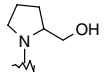
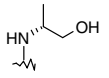
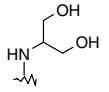
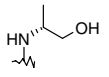
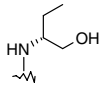
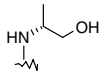
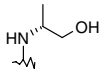
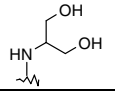
Hydrogenolysis of **19**–**20** with sodium in liquid ammonia[11] gave the thiols **21**–**22**. Subsequent treatment of **21**–**22** with sodium hydride, followed by reaction with a range of activated halides, gave an array of sulfides **25**–**34**. The second approach involved reaction of the benzylsulfones **23**–**24** prepared by oxidation of **19**–**20** with Oxone®, with a range of thiols. Due to the propensity of thiols to form disulfides, the optimised conditions involved treatment of sulfone with a thiol under an inert atmosphere, using degassed solvent, and in the presence of a stoichiometric quantity of sodium borohydride.

Previous SAR studies around the 7-position had shown that a hydroxylated alkylamino substituent is beneficial for potency. In our follow up programme based on this structural knowledge we treated compound **2** in a combinatorial manner with a designed set of amines in order to explore the favoured steric and electronic parameters of this motif, Scheme 1. From over 290 compounds synthesised, only compounds that had a hydroxyethyl amino core had binding affinity (pIC_{50}) of >7.0 Table 2. Further hydroxyl substitution of the alkyl side chain is also tolerated as exemplified by the diols **3**–**5**. Exploration of substitution upon the hydroxyethyl amino group showed that small alkyl groups are preferred, compare, for example, the (*R*)-alaninol derivative **6** with **8**, giving the alaninol series a 6-fold advantage in potency over **1**. Moreover comparison of **6** with **7** shows a 100-fold preference for (*R*) enantiomer over the (*S*). Introduction of a secondary cyclic amine at the 7-position was less well tolerated as shown by **9,** a cyclic analogue of **6**.

Turning our attention to varying both the 2- and 7-substituents using a 2-dimensional matrix via the tandem displacement protocol, the results further reinforced the importance of substitution in the 7-position by hydroxyalkyl amines. Investigation of the substituent at the 2-position showed that amino was most preferred, small secondary alkyl amines, for example, methyl **16**, isopropyl **14**–**15** and cyclopropyl **12**–**13**, led to a 2- to 5-fold drop in potency and small tertiary amines **17**–**18** a 100-fold drop when compared to **6**. The (*R*)-alaninol substituent was found to confer most potency regardless

Table 2. Substitution at the 2- and 7-positions



	Y	X	CXCR2 Binding[a] pIC_{50}
3		NH_2	7.8
4		NH_2	7.1
5		NH_2	7.5
6		NH_2	8.4
7		NH_2	7.2
8		NH_2	7.6
9		NH_2	<6.0
12			7.7
13			7.0
14			7.5
15			7.0
16			8.1
17			6.4
18			5.8

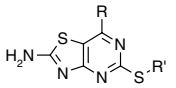
[a] Binding affinity was determined via a scintillation proximity assay (SPA) using [^{125}I]IL-8 and human recombinant CXCR2 (hrCXCR2) receptor expressed in HEK 293 membranes.[7]

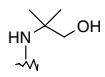
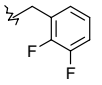
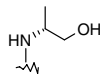
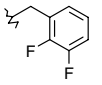
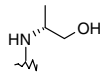
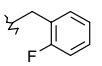
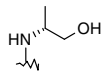
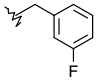
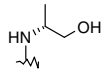
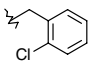
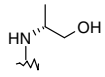
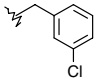
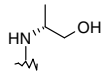
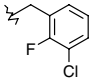
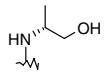
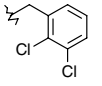
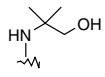
of the 2-substituent. Variation to the 5-position was undertaken by one of two synthetic routes, reaction of the thiols **21**–**22** with a range of benzyl and heteroaromatic halides, or through the treatment of the sulfones **23**–**24** with thiolates Scheme 2. Screening results gave good correlation between the measured binding affinity of the compound with substituent size and substitution pattern, with a preference for small substituents at the ortho and/or meta positions, of such groups lipophilic and electron withdrawing substituents were the most potent Table 3, fluorine and chlorine being most preferred.

There was an increase in potency observed for 2,3-disubstitution with fluorine over both ortho and meta substitution, compare **6**–**25** and **26**, however this did not transfer to the 2,3-dichloro analogue **30**. Combining the substitution of an ortho fluoro with a meta chloro **29** gave a compound equipotent with **6**. Replacement of the benzyl moiety with heteroaromatic rings is generally less well tolerated. Again best potency is observed when the 7-position is substituted by the (*R*)-alaninol motif as shown by **31**–**34**.

In summary, our SAR investigation designed to optimise all three substitution positions on compound **1**

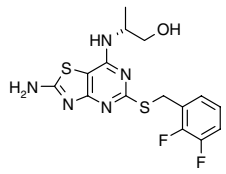
Table 3. Substitution at the 5-position



	R	R′	CXCR2 Binding[a] pIC_{50}
1			7.6
6			8.4
25			7.9
26			7.8
27			7.5
28			7.9
29			8.4
30			7.8
31			7.0
32			7.5
33			6
34			6.9

[a] Binding affinity was determined via a scintillation proximity assay (SPA) using [^{125}]IL-8 and human recombinant CXCR2 (hrCXCR2) receptor expressed in HEK 293 membranes.[7]

Table 4. Profile of thiazolopyrimidine **6**



Assay	Thiazolopyrimidine **6**
Binding CXCR2 pIC_{50}	8.4
Functional pA2	9.0
Human plasma protein binding %	97.4
Solubility (pH 7.4) μg/ml	1.5
Rat iv Cl ml/min/Kg	17
Rat iv V_{ss} L/Kg	1.03
Rat iv $T_{1/2}$ h	1.2
Hu CaCo2 mean A to B cm/s $\times 10^6$	2.47
Hu CaCo2 mean B to A cm/s $\times 10^6$	13.68
Mean efflux ratio	5.5
Rat po bioavailability %	9

led to the identification of **6,** a more potent CXCR2 receptor antagonist. This was achieved in part through the development of a novel tandem displacement reaction allowing the opportunity to rapidly explore two points of diversity on the thiazolopyrimidine heterocycle. A key finding from this programme of work highlighted that the (*R*)-alaninol substituent is the best 7-substituent regardless of the variations at the 2- and 5- positions. The in vitro metabolic stability and in vivo pharmacokinetics were determined for **6** and the results are shown in Table 4. Compound **6** is more stable in vivo and less plasma protein bound than **1,** however the rat oral bioavailability of **6** is lower, which could be attributed to a poorer absorption due to the decrease in its measured aqueous solubility, and/or the possibility of being a substrate for the efflux transporter *P*-glycoprotein (pGp). CaCo2 permeability measurements showed a modest but significant efflux component with a mean efflux ratio of ~6, which was not completely reduced in the presence of verapamil a known competitive inhibitor of pGp. Investigation of replacements for the thiazolopyrimidine heterocycle with a view to addressing the factors influencing the low bioavailability will form the basis of a new programme of work.

References and notes

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8. Synthesis of compound **6**: A mixture of **2** (0.1 g) and (2*R*)-2-amino-propan-1-ol (0.5 g) in a solution in tetrahydrofuran (10 ml) was heated in a sealed vessel at 100 °C for 18 h. The mixture was evaporated to dryness and purified (HPLC, Novapak® C18, 0.1% aqueous ammonium acetate:acetonitrile, gradient elution 70:30 to 0–100 over 15 min) to afford **6** 0.051 g (46%) as a colourless solid. MS (APCI) 384 (M+H, 100%); ^{1}H NMR (399.98 MHz, DMSO) δ 8.03 (s, 2 H), 7.40–7.26 (m, 2 H), 7.17–7.10 (m, 1 H), 7.01 (d, J = 7.9 Hz, 1H), 4.70 (s, 1H), 4.39 (dd, J = 18.5, 14.4 Hz, 2H), 4.19 (quintet, J = 6.5 Hz, 1H), 3.44 (q, J = 5.0 Hz, 1H), 1.10 (d, J = 6.7 Hz, 3H); ^{13}C NMR (100.585 MHz, DMSO) δ 170.88 (s), 168.70 (s), 164.85 (s), 155.19 (s), 149.61 (dd J_{CF} = 245, 12 Hz), 148.04 (dd, J_{CF} = 246, 12 Hz, 128.63 (d, J_{CF} = 11.5 Hz), 126.06 (t, J_{CF} = 3 Hz), 124.26 (dd, J_{CF} = 5,7 Hz),115.97 (d, J_{CF} = 17 Hz), 98.09 (s), 64.27 (s), 47.97 (s), 27.01 (s), 17.26 (s).
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