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Hit-to-Lead studies: The discovery of potent, orally bioavailable thiazolopyrimidine CXCR2 receptor antagonists

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Abstract—A Hit-to-Lead optimisation programme was carried out on a high throughput screening hit, the thiazolopyrimidine **1**, resulting in the discovery of the potent, orally bioavailable CXCR2 antagonist **29**.

The use of high throughput screening (HTS) and Hit-to-Lead (HtL) is now widespread in the pharmaceutical industry.[1] The lead identification process has been more accurately defined as two distinct steps—Active-to-Hit (AtH) and Hit-to-Lead.

AtH and HtL are based on our definitions of Actives, Hits and Leads.

- *Actives* are defined as being the raw output from a HTS and will be in the form of an IC_{50} using a HTS assay on a solution in DMSO of a sample from our compound collection.
- *Hits* are defined in terms of a profile that includes confirmation of chemical structure and biological activity as well as physical chemistry data and drug metabolism and pharmacokinetic (DMPK) measurements. DMPK data are used at this early stage of the drug discovery process to avoid failure at a late stage due to poor pharmacokinetics (metabolic instability and/or poor oral absorption).
- *Leads* are described in terms of a target profile of chemical and biological data. This profile was chosen so that a Lead Optimisation (LO) programme would have a reasonable chance of producing a Candidate Drug (CD) in a target time of two years.

The Lead target profile used is shown in Figure 1. The potency target was arrived at by the empirical observation that once the 100 nM level has been achieved then further increases in potency to the nanomolar level are normally possible. The physicochemical parameters are a reflection of the lipophilicity and molecular weight increases normally seen in LO programmes and are based on an extension of the arguments put forward by Teague et al.,[2] i.e., lower than the molecular weight (<500) and lipophilicity ($\log P < 5$) drug-like targets outlined by Lipinski et al.[3] In vitro and in vivo DMPK targets have been equated to clearances of approximately half liver blood flow, detectable oral bioavailability and non-limiting plasma protein binding. In this way, lead compounds should give some oral exposure to aid an in vivo hypothesis test of the target mechanism and be a solid starting point for a LO project leading to an orally active CD. Lead compound should fulfil the majority of the lead criteria—an explanation of any missed targets should be provided. It is also important at the lead stage that the series has a large scope for further optimisation including areas of unexplored SAR that will enable the LO project to increase potency and improve other properties going forward to CDs. HTS and HtL should aim to provide multiple series at the lead stage and this is seen as an important factor in de-risking future LO work. In this way, consistent standards are maintained for entry into LO and this should provide compound series that have optimisable potency and DMPK.

In our previous paper on the HtL carried out on triazolethiols,[4] the biological background and rationale in disease were presented. In this paper, we present a HtL study that was carried out on a chemically distinct

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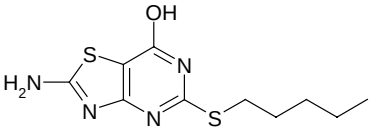
Potency	$IC_{50} < 0.1\mu M$
Rat Hepatocytes	clearance < 14μL/min/10^6 cells
Human Liver Microsomes	clearance < 23μL/min/mg
Rat iv PK	clearance < 35mL/min/kg, Vss > 0.5L/kg, $T_{1/2} > 0.5$hr
Oral Bioavailability	F > 10%, PPB < 99.5%
Physical Chemical	MWt < 450, clogP < 3.0, logD < 3.0
Additionally: Clear SAR, Appropriate selectivity data and Patentable, Multiple series	

Figure 1. Hit-to-Lead generic Lead target profile.

Table 1. Hit profile of thiazolopyrimidine **1**

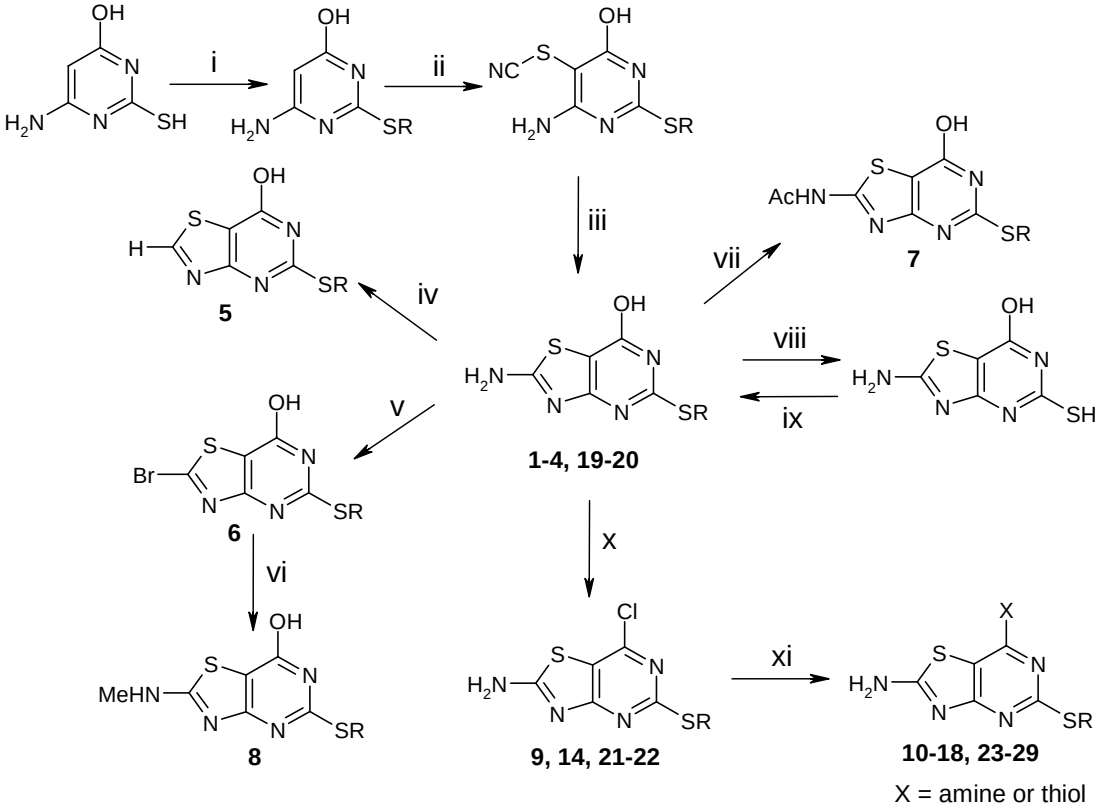


Generic lead criteria[a]	Compound **1**
Binding $IC_{50} < 0.1$ μM	10 μM
Ca flux $IC_{50} < 0.1$ μM	2.0 μM
Rat hepatocyte Cl < 14	49
Human microsome Cl < 23	18
Molecular weight < 450	270
Solubility > 10 μg/mL	27
c log *P* < 3.0	2.0
log *D* < 3.0	2.9

[a] Units as Figure 1 where not stated.

hit compound to provide a second lead series of CXCR2 antagonists. A HTS was undertaken to identify compounds that blocked the binding of [^{125}I]IL8 to human recombinant CXCR2 (hrCXCR2) expressed in HEK 293 membranes using a scintillation proximity assay (SPA). Subsequently at the AtH stage, compounds with binding inhibition seen in the HTS SPA were initially validated in a more conventional filter wash hrCXCR2 [^{125}I]IL8 binding assay. Confirmation of functional antagonism was shown by blockade of GROα stimulated intracellular calcium mobilisation in isolated human neutrophils using a fluorescence imaging plate reader (FLIPR).[5]

The thiazolopyrimidine **1** was one of the hits from the CXCR2 HTS. This novel compound was part of our compound collection and, while having weak activity in the binding assay (IC_{50} 10 μM), had comparable potency in a functional FLIPR assay (IC_{50} 2.0 μM). The profile of this CXCR2 hit is shown in Table 1 compared with the generic lead target profile. Our plan was to examine in turn the three thiazolopyrimidine substituents looking for SAR leading to increasing potency. The poor in vitro rat hepatocyte clearance



Scheme 1. Reagents and conditions: i—RBr, NaOH, EtOH; ii—KSCN, pyridine, DMF, 65 °C then cool to 5 °C, add Br_2, 2 h; iii—DMF, H_2O, 10 h, 120 °C; iv—*t*-BuONO, THF, 65 °C; v—TMSBr, *t*-BuONO, CH_3CN, rt, 16 h; vi—$MeNH_2$, MeOH, rt; vii—AcCl, pyridine, CH_2Cl_2; viii—$AlBr_3$, toluene, 60 °C, 6 h; ix—RBr or RI, *t*-BuOK, DMSO, 3 days, rt; x—$POCl_3$, $PhNEt_2$, reflux, 2 h; xi—amine or thiol, THF or DMSO, 65 °C.

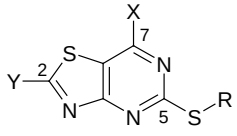
was associated with possible conjugation of the hydroxyl group and the presence of the lipophilic *S*-pentyl was also seen as a site of potential metabolic instability.

The synthetic routes to the thiazolopyrimidines prepared in this paper are shown in Scheme 1. Alkylation of 6-aminothiouracil followed by thiocyanation and cyclisation[6] gave the alkylthiothiazolopyrimidines **1**–**4**. Diazotisation with *tert*-butyl nitrite in THF or in the presence of TMSBr gave the 2-protio- and 2-bromothiazolopyrimidines **5** and **6**. Acetylisation of **1** gave **7** and reaction of the bromo compound **6** with methylamine gave the methylamino analogue **8**. The hydroxy on thiazolopyrimidines **1** and **4** was converted to the chloro (compounds **9** and **14**) using phosphorus oxychloride. This chloro was readily displaced by a variety of amines and thiols to give compounds **10**–**13** and **15**–**18**. In order to vary the 5-alkylthio more widely we prepared the thiol by de-benzylation of **4** with aluminium tribromide.[7] This thiol was then re-alkylated to give **19** and **20**, which were chlorinated as above to give **21** and **22**. Reaction of the chloro compounds **14**, **21** and **22** with the appropriate hydroxyamines gave the compounds **23**–**29**.

The initial focus of HtL was to examine the effects of simple changes to the substituents on the thiazolopyrimidine core. Variation of the *S*-alkyl 5 substituent was undertaken via synthesis of each analogue starting from aminothiouracil (Scheme 1). CXCR2 antagonist activity (Table 2) was lost in the change from pentyl (**1**) to methyl (**2**) and ethyl (**3**). An encouraging increase in potency was seen though with the benzylthio analogue **4**. Some simple transformations were undertaken on the 2-amino group. Only replacement by hydrogen (**5**) showed any activity, bromo (**6**), acetylamino (**7**), and methylamino (**8**) were all inactive. The 2-amino group was kept constant in all subsequent analogues prepared in HtL. The hydroxyl in the 7 position is amenable to variation via the readily prepared chloro compounds **9** and **14**. Displacement with a wide variety of amines was undertaken in a combinatorial experiment. Compounds that showed activity as well as two inactive analogues were re-synthesised as solid characterised compounds. Simple amino substituents such as diethylamino (compound **10**) and cyclopentylamino (compound **11**) were inactive, amino (compound **18**) had reduced potency but hydroxyethylamino (compound **12**) retained activity. This activity was sensitive to chain length as the hydroxypropyl analogue (**13**) was inactive. Sulfur linked analogues (**15**–**17**) were also inactive. This preliminary examination of SAR indicated that areas worthy of urther exploitation were the 5-benzylthio and the 7-hydroxyethylamino substituents.

Variation of the 5-benzyl substitution pattern was undertaken combinatorially using the reaction of the 7-hydroxy-5-thiol with a variety of benzyl halides. Two substitution patterns were chosen as they gave increased potency, the 3-phenoxy and 2,3-difluoro. Confirmation (Table 3) was obtained by the resynthesis of

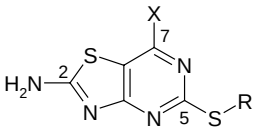
Table 2. CXCR2 antagonist binding potencies



	X	Y	R	CXCR2 IC_{50} (μM)[a]
1	OH	NH_2	Pentyl	10
2	OH	NH_2	Me	NA
3	OH	NH_2	Et	NA
4	OH	NH_2	CH_2Ph	1.3
5	OH	H	Pentyl	10
6	OH	Br	Pentyl	NA
7	OH	AcNH	Pentyl	NA
8	OH	MeNH	Pentyl	NA
9	Cl	NH_2	Pentyl	NA
10	NEt_2	NH_2	Pentyl	NA
11	NH-Cyclopentyl	NH_2	Pentyl	NA
12	$NH(CH_2)_2OH$	NH_2	Pentyl	7
13	$NH(CH_2)_3OH$	NH_2	Pentyl	NA
14	Cl	NH_2	CH_2Ph	NA
15	SCH_2CO_2Me	NH_2	CH_2Ph	NA
16	SCH_2CO_2H	NH_2	CH_2Ph	NA
17	$S(CH_2)_2OH$	NH_2	CH_2Ph	NA
18	NH_2	NH_2	CH_2Ph	10

[a] NA = <50% inhibition at 10 μM.

Table 3. CXCR2 antagonist binding potencies



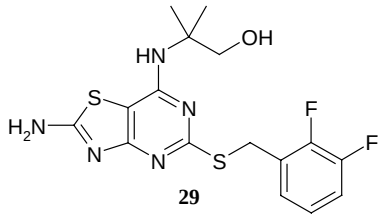
	Compound, CXCR2 IC_{50} (μM)[a]		
	R = $PhCH_2$	R = (3-PhOPh)CH_2	R = (2,3-DiFPh)CH_2
X = OH	**4**, 1.3	**19**, 0.48	**20**, 0.27
X = Cl	**14**, NA	**21**, NA	**22**, NA
X = (*S*)-$NHCH(Et)CH_2OH$	**23**, 3.9	**25**, 1.4	
X = (*R*)-$NHCH(Et)CH_2OH$	**24**, 0.12	**26**, 0.35	**28**, 0.0095
X = $NHC(Me)_2CH_2OH$		**27**, 0.050	**29**, 0.014

[a] NA = <50% inhibition at 10 μM.

Table 4. Thiazolopyrimidine DMPK data

Compound	Rat hepatocyte Cl (μL/min/10^6 cells)	Human microsome Cl (μL/min/mg)	Rat pharmacokinetics iv and po			
			Cl (mL/min/kg)	V_{ss} (L/kg)	$T_{1/2}$ (h)	F (%)
1	49	18				
4	68	<3				
20	3	2	9.5	0.9	3.5	
24	14	6	40	2.2	1.0	
29	5	31	25	1.9	1.2	15

Table 5. Lead profile of thiazolopyrimidine **29**



29

Generic lead criteria[a]	Compound **29**
Binding IC_{50} < 0.1 μM	0.014 μM
Ca flux IC_{50} < 0.1 μM	0.04 μM
Rat hepatocyte Cl < 14	4
Human microsome Cl < 23	31
Rat iv Cl < 35	25
Rat iv V_{ss} > 0.5 L/kg	1.9
Rat iv $T_{1/2}$ > 0.5 h	1.2
Rat po bioavailability > 10%	15%
Plasma protein binding < 99.5%	98.4%
Molecular weight < 450	397
Solubility > 10 μg/mL	0.5
clog P < 3.0	3.1
log D < 3.0	3.4

[a] Units as Figure 1 where not stated.

solid, characterised samples of compounds **19** (IC_{50} 0.48 μM) and **20** (IC_{50} 0.27 μM). The chloro compounds (**14**, **23** and **24**) were then reacted with a set of 2-hydroxyamines in a combinatorial experiment. A large number of alkyl substituted hydroxyamines are readily available in chiral form as they are derived from amino acids. The most potent of these were chosen for resynthesis together with the optical isomer. Comparison of compounds **19** and **25** with **20** and **26** shows the chiral preference for (*R*) over (*S*) in this substituent. Compounds **28** and **29** were the most potent prepared in this study.

In vitro metabolic stability and in vivo pharmacokinetics were determined for key compounds throughout this study. The results are shown in Table 4. The initial pentylthio compound **1** was metabolised in vitro by rat hepatocytes indicating a conjugation mechanism. The benzylthio analogue **4** has a similar profile but the difluorobenzyl compound **20** is much more stable to both rat hepatocytes and human microsomes. This indicated that oxidation followed by conjugation was probably the metabolic route and that could be blocked by fluorination of the phenyl ring. This profile is maintained in vivo in the rat, compound **20** having low clearance and adequate intravenous half-life. A slight increase in metabolic instability is seen with the hydroxyamino derivatives **24** and **29** but these compounds otherwise have an encouraging profile. In particular, compound **29** (Table 5) fulfils most of the lead DMPK criteria including oral bioavailability even though the solubility is low. A slight increase in human microsome clearance was observed but other close analogues (**20** and **24**) were more stable. The thiazolopyrimidines exemplified by compound **29** formed the basis of a new Lead Optimisation project.

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