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Hit-to-Lead Studies: The Discovery of Potent, Orally Bioavailable Triazolethiol CXCR2 Receptor Antagonists

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Abstract—A Hit-to-Lead optimisation programme was carried out on the high throughput screening hit, the triazolethiol **1**, resulting in the discovery of the potent, orally bioavailable triazolethiol CXCR2 receptor antagonist **45**.

The use of High Throughput Screening (HTS) is now widespread in the pharmaceutical industry. There was an expectation that, once a screen was established for a particular target, then potent lead compounds or candidate drugs would be found. The reality is often far from this. Bridging the gap between the end of a HTS and the start of a full Lead Optimisation (LO) project has been described as Hit-to-Lead (HtL).[1] Hits from HTS are profiled and compared to a generic target lead criteria. The lead target profile used is shown in Figure 1. Lead series then have a balance of properties — potency and SAR an encouraging metabolic and selectivity profile—such that rapid (less than 2 years) further optimisation should provide Candidate Drugs (CDs).

Chemokines play an important role in immune and inflammatory responses in various diseases and disorders, including asthma and allergic disease, as well as autoimmune pathologies such as rheumatoid arthritis and atherosclerosis. These small secreted molecules are a growing superfamily of 8–14 kDa proteins characterised by a conserved four-cysteine motif. The chemokine superfamily can be divided into two main groups exhibiting characteristic structural motifs, the Cys-X-Cys (CXC) and Cys-Cys (CC) families.[2] The CXC chemokines include several potent chemoattractants and activators of neutrophils such as interleukin-8 (IL8), GROα and neutrophil activating peptide 2 (NAP2).

Potency $IC_{50} < 0.1\mu M$
+ Confirmatory whole cell activity
Rat Hepatocytes clearance < 14μL/min/10^6 cells
Human liver Microsomes clearance < 23μL/min/mg
Rat iv Clearance < 35mL/min/kg, Vss > 0.5L/kg,
$T_{1/2}$ > 0.5hr, Rat po Bioavailability F > 10%
Plasma protein binding < 99.5%, solubility >10μg/mL
Molecular Weight < 450, clogP < 3.0, logD < 3.0

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

Studies have demonstrated that the actions of the chemokines are mediated by subfamilies of G protein-coupled receptors, among which are the receptors designated CCR1 to CCR10 and CXCR1 to CXCR5. These receptors represent good targets for drug development since agents which modulate these receptors would be useful in the treatment of disorders and diseases such as those mentioned above. The CXCR2 receptor is one of the receptors that IL8 activates and antagonists of this receptor should find particular use in the treatment of a number of diseases.

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 hit evaluation stage compounds with binding inhibition seen in the HTS SPA assay were initially validated in a more conventional filter wash

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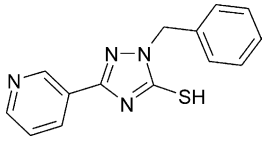
hrCXCR2 [^{125}I]-IL8 binding assay. Confirmation of functional antagonism was shown by blockade of GROα stimulated intracellular calcium mobilisation in isolated human neutrophils using a Fluorescence Imaging Plate Reader (FLIPR).[3]

The triazole **1** emerged inter alia from the CXCR2 HTS and, while having only modest potency in the binding assay (IC_{50} 4.6 μM), had demonstrable and comparable functional activity in the FLIPR assay (IC_{50} 2.4μM). The profile of this CXCR2 hit is in Table 1 compared with the generic lead target profile. The DMPK profile was satisfactory at this stage and HtL focused on exploring SAR to increase potency and assessing the importance of the thiol-thione functionality.

Table 1. Hit profile of triazolethiol **1**

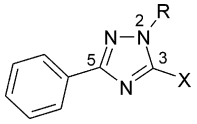


Generic lead criteria[a]	Triazolethiol **1**
Binding $IC_{50}<0.1$ μM	4.6 μM
Ca Flux $IC_{50}<0.1$ μM	2.4 μM
Rat hepatocyte Cl < 14	19
Human microsome Cl < 23	13
Rat iv Cl < 35	12
Rat iv Vss > 0.5 L/kg	1.3
Rat iv $T_{1/2}>0.5$ h	3.4
Molecular weight < 450	268
ClogP < 3.0	3.4

[a]Units as Figure 1 where not stated.

Replacement of the pyridinyl ring by phenyl (compound **2**)[4] gave a slightly more potent analogue which was the starting point for variation of the thiol (Table 2). Analogues with hydroxy (**3**), amino (**4**), and without the thiol (**5**) were prepared using literature methods;[5–7] all were inactive as CXCR2 antagonists. Methylation of the thiol (**6**) and acetylation (**7**) and benzenesulphonylation (**8**) of the amino also gave inactive compounds. It is assumed that the acidic nature of the thiol (pK_a ~6.5) and its size and steric environment are essential for receptor binding as smaller or sterically demanding acidic groups (OH, NHAc and $NHSO_2Ph$) are inactive. All subsequent analogues prepared in HtL preserved the triazolethiol structure.

Table 2. CXCR2 antagonist binding potencies

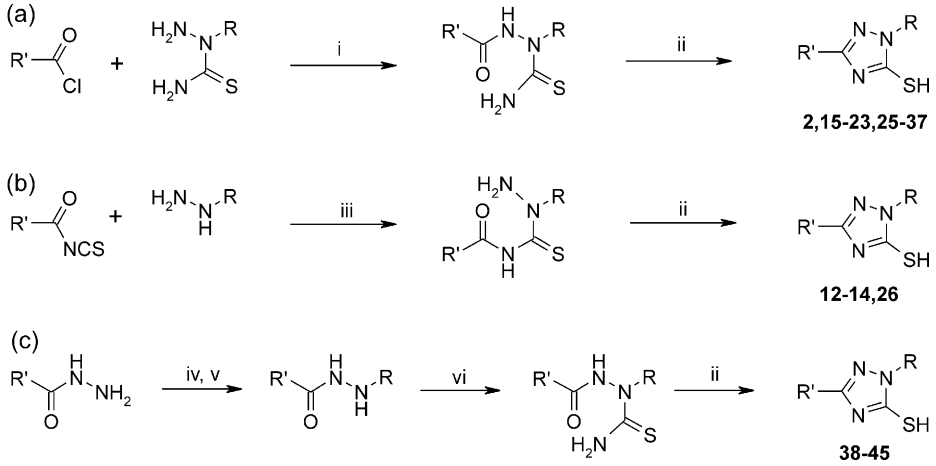


	R	X	CXCR2 IC_{50} (μM)[a]
2	$PhCH_2$	SH	2.4
3	$PhCH_2$	OH	NA
4	$PhCH_2$	NH_2	NA
5	$PhCH_2$	H	NA
6	$PhCH_2$	SMe	NA
7	$PhCH_2$	NHAc	NA
8	$PhCH_2$	$NHSO_2Ph$	NA

[a]NA, < 50% inhibition at 10 μM.

Next variation of the 2-benzyl substituent was investigated. Published routes to these compounds utilise the reaction of 2-substituted thiosemicarbazides with acid chlorides,[4] or acylisocyanates with hydrazines[7] followed by cyclisation (Scheme 1a and b). The compounds **9**–**14** in Table 3 were prepared from commercially available starting materials and offered no improvements in potency over 2-benzyl. Phenyl, methyl and hydrogen were all inactive and only the 3-hydroxybenzyl analogue **14** had any activity. The substitution on the 5-phenyl was investigated next, keeping the 2-benzyl constant, using the route described above. Table 3 lists the compounds prepared (**15**–**37**) and their activity. Various substitutions were seen as potency enhancing, in particular 2-chloro (**34**), 2,4-dichloro (**35**), 2-bromophenyl (**36**) and 2,3-dichloro (**37**) gave 10-fold increases in potency compared to the unsubstituted phenyl (**2**).

In order to look for further variations of substituents on the 2-benzyl group, it was necessary to find an alternative route that would allow simpler starting materials

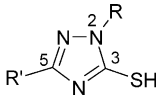


Scheme 1. (i) Pyridine, 16 h, rt; (ii) 1 M $NaHCO_3$, 16 h, 100 °C; (iii) Et_3N, toluene, 1 h, 80 °C; (iv) aldehyde, MeOH/HCl; (v) Et_3SiH, TFA, 0 °C; (vi) EtOH/HCl, NH_4SCN, 18 h, 75 °C.

to be used. Benzylation of the 3-H compound (**11**) proceeds first on sulphur and a di-benzylation followed by *S*-debenzylation route did not work in our hands. The route followed[6] is shown in Scheme 1c. Substituted aldehydes were condensed with benzoylhydrazides to give the corresponding hydrazones. These hydrazones were then reduced using triethylsilane in trifluoroacetic acid to give the substituted benzoylhydrazines. Reaction with ammonium thiocyanate in hot ethanol containing hydrogen chloride gave the intermediate semicarbazides, which were cyclised in hot sodium bicarbonate solution to give the required triazolethiols (**38–45**). A number of analogues were prepared keeping the 5-substituent constant as 2,4-dichlorophenyl; most potent of these was the 3-chlorobenzyl having IC_{50} 0.092 μM. Checking back to other potent 5-substituents led to the preparation of the lead compound, the 2-(3-chlorobenzyl)-5-(2-chlorophenyl) analogue (**45**) (Table 3).

Some preliminary SAR conclusions can be drawn on the basis of the compounds prepared in this paper. The substitution patterns, orientation in space and presence of the two phenyl rings is seen as important. In the 5-position of the triazole (Table 3), cyclohexyl (**15**) and methyl (**16**) were inactive whilst almost all aromatic groups tried had some antagonist activity. Phenyl and thiophene (**2** and **26**) were more potent than pyridyl and furan (**19** and **20**). In general it was found that analogues with a 2-substituted phenyl were the most potent and that the preferred substituent was chlorine or bromine. 2-Chlorophenyl became the 5-substituent of choice. 2-Chloro (**34**) was better than 2-fluoro (**30**), 2-methoxy (**28**) or 2-methyl (**27**) and 2-hydroxy (**18**) was inactive possibly due to an internal hydrogen bond with the triazole. 2-Chloro was preferred to 2-bromo because of reduced lipophilicity and better pharmaceutical acceptability. Some of the potency enhancement could be lipophilicity driven but the 2-chloro substituent would be expected to cause a twist out of plane between the phenyl and triazole. With a single substituent this could be up to 30° without loss of resonance between the rings. In general, addition of 3- or 4-substituents did not cause appreciable potency changes; for example. compare 2-chloro (**34**) with 2,3-, 2,4-, 2,5-dichloro (**37**, **35**, **33**). Optimisation at the 2-position was undertaken in two separate series (5-phenyl analogues **2**, **9–14**) and 5-(2,4-dichlorophenyl) analogues **35**, **38–44**). Initially only benzyl (**2**) gave any activity, phenyl, methyl and hydrogen being inactive (**9–11**). Activity was removed by the addition of 2-chloro (**13**) and 4-trifluoromethyl (**12**), only 3-hydroxy (**14**) maintained activity. The preference for a 3-substituent was confirmed and extended in the more potent 5-(2,4-dichlorophenyl) series. 3-Chloro (**44**) gave an increase in potency compared to the unsubstituted compound (**35**), other 3- and 4-substitutuents were less active (**38–43**). One interesting observation was that the phenethyl analogue (**41**) had similar potency to benzyl (**35**).

Table 3. CXCR2 antagonist binding potencies



	R	R′	CXCR2 IC_{50} (μM)[a]
2	$PhCH_2$	Ph	2.4
9	Ph	Ph	NA
10	Me	Ph	NA
11	H	Ph	NA
12	4-CF_3PhCH_2	Ph	NA
13	2-$ClPhCH_2$	Ph	NA
14	3-$OHPhCH_2$	Ph	4.4
15	$PhCH_2$	Cyclohexyl	NA
16	$PhCH_2$	Me	NA
17	$PhCH_2$	4-MePh	NA
18	$PhCH_2$	2-OHPh	NA
19	$PhCH_2$	4-Pyridinyl	7.7
20	$PhCH_2$	2-Furanyl	4.2
21	$PhCH_2$	4-CNPh	3.5
22	$PhCH_2$	3-CF3Ph	3.5
23	$PhCH_2$	4-CF3Ph	2.8
24	$PhCH_2$	4-MeOPh	2.3
25	$PhCH_2$	3,5-DiClPh	2.0
26	$PhCH_2$	2-Thienyl	2.0
27	$PhCH_2$	2-MePh	1.4
28	$PhCH_2$	2-MeOPh	1.4
29	$PhCH_2$	3-ClPh	1.0
30	$PhCH_2$	2-FPh	0.89
31	$PhCH_2$	4-ClPh	0.83
32	$PhCH_2$	3,4-DiClPh	0.80
33	$PhCH_2$	2,5-DiClPh	0.67
34	$PhCH_2$	2-ClPh	0.45
35	$PhCH_2$	2,4-DiClPh	0.41
36	$PhCH_2$	2-BrPh	0.35
37	$PhCH_2$	2,3-DiClPh	0.35
38	4-$MeOPhCH_2$	2,4-DiClPh	10
39	3-$MeOPhCH_2$	2,4-DiClPh	4.2
40	3-$MePhCH_2$	2,4-DiClPh	0.73
41	$PhCH_2CH_2$	2,4-DiClPh	0.45
42	4-$ClPhCH_2$	2,4-DiClPh	0.30
43	3-$PhOPhCH_2$	2,4-DiClPh	0.17
44	3-$ClPhCH_2$	2,4-DiClPh	0.092
45	3-$ClPhCH_2$	2-ClPh	0.028

[a]NA, <50% inhibition at 10 μM.

Investigation of the role of the central triazole ring was also undertaken. A number of heterocycles substituted with phenyl and benzyl were obtained from the corporate compound collection and from external suppliers but none showed any activity (data not shown). Limited variation of the 3-position on the triazole was carried out but only 3-thiol had any activity (**2** vs **3–8**). Initially the acidic nature of the thiol (pK_a 6.20) was thought to be important. This trend has been noted by others; SB225002[8] and other urea based CXCR2 antagonists[9] are all acidic in nature (Fig. 2). The role of the anionic group has been investigated further by this group,[10] finding that alteration of the phenolic pK_a to give uncharged molecules gave greatly reduced potency. In the case of the triazoles though more subtle SAR exists. The hydroxy compound **3** has appreciable ionised form (pK_a 7.80) and the sulphonamide **8** (pK_a 6.71) is even more acidic but both lack activity. With the sulphonamide, the presence of a large extra substituent might be

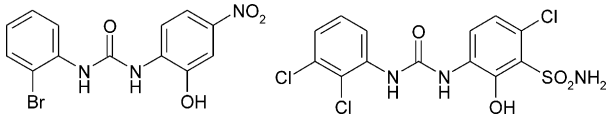
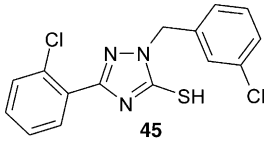


Figure 2. Structure of acidic urea CXCR2 antagonists.

expected to interfere with binding to the receptor but at present there is no explanation of the lack of activity of the hydroxy compound **3**.

Table 4. Lead profile of triazolethiol **45**



45

Generic lead criteria[a]	Triazolethiol **45**
Binding $IC_{50} < 0.1$ μM	0.028 μM
Ca flux $IC_{50} < 0.1$ μM	0.048 μM
Rat hepatocyte Cl < 14	26
Human microsome Cl < 23	14
Rat iv Cl < 35	12
Rat iv Vol > 0.5 L/kg	8
Rat iv $T_{1/2} > 0.5$ h	9
Rat po bioavail. > 10%	61%
Plasma prot. bind. < 99.5%	99.0%
Molecular weight < 450	336
Solubility > 10 μg/mL	20
clogP < 3.0	4.3
Log D < 3.0	3.2

[a]Units as Figure 1 where not stated.

The profile of the lead compound (**45**) is shown in Table 4 compared with the lead target profile. Compound (**45**) has good lead-like potency, both binding and functional in the calcium flux assay, and the in vitro rat hepatocyte and human microsome data was acceptable. It is a weak acid (pK_a 6.4) and has borderline lipophilicity and this is reflected in the high plasma protein binding. However the compound has maintained the satisfactory in vivo DMPK profile having a 9 h intra venous half-life and over 60% oral bioavailability. The SAR presented clearly shows scope for further improvement in potency by looking for additional interactions via further aromatic substituents and alteration of linker group between the triazole and phenyl group in the 2-position. Selectivity data was acceptable and analogues with substituents on both aromatic rings are novel. The triazolethiols exemplified by compound **45** formed the basis of a new LO project.

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