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Imidazolylpyrimidine based CXCR2 chemokine receptor antagonists

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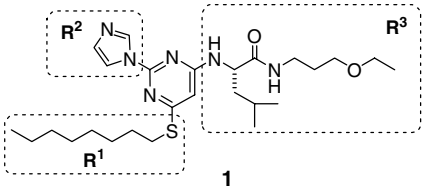
Abstract—An imidazolylpyrimidine was identified in a CXCR2 chemokine receptor antagonist screen and was optimized for potency, in vitro metabolic stability, and oral bioavailability. It was found that subtle structural modification within the series affected the oral bioavailability. Potent and orally available CXCR2 antagonists are herein reported.

Chemokines,[1] by interaction with their respective G-protein-coupled receptor (GPCR), have a profound influence on leukocyte migration and upregulation of adhesion receptors. They are predominantly classified into two large families, CXC and CC, based on their amino acid sequence. The actions of CXC chemokines are mediated through six cell surface receptors, CXCR1 to CXCR6.

Ligands for the CXCR2 receptor, for example, interleukin-8 (IL-8, CXCL8), induce migration of neutrophils, monocytes, and T lymphocytes toward a site of inflammation. As such, the CXCR2 receptor and IL-8 are thought to play a crucial role in inflammatory diseases. Therefore, antagonism of CXCR2 could be beneficial to these inflammatory diseases.[2]

The goal was to identify small molecule antagonists of human CXCR2 that possessed suitable potency and pharmacokinetic properties for proof-of-principle efficacy studies.

A binding assay based on membranes prepared from CHO cell transfected with human CXCR2 receptor and labeled IL-8 was used to screen the Pharmacopeia libraries (~500,000 compound, 1996). The initially active compound identified, imidazolylpyrimidine **1**, had a K_i of 60 nM and it served as the starting point for an optimization program. Compound **1** was divided into four domains: the pyrimidine core and three pendant groups R^1, R^2, and R^3. Presented herein is the optimization of the R^1 and R^2 domains.



1
Ki = 60 nM

Preparation of the pyrimidine analogs is exemplified for compound **40** (Scheme 1). 2,4-Dichloro-6-propylpyrimidine (**4**) was prepared from reaction of 2,6-dichloropyr-

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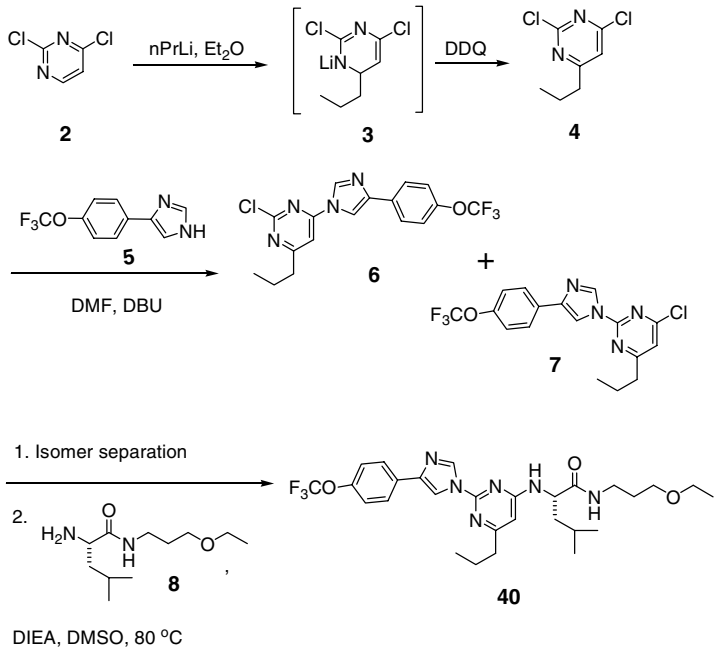
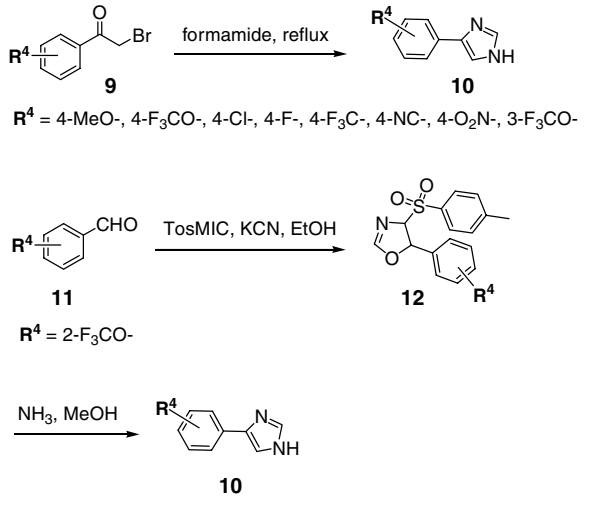


Scheme 1. Preparation of pyrimidine analogs.

imidine (**2**) with *n*-propyllithium followed by oxidation of the intermediate (**3**) with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).[3] Reaction of **4** with 4-trifluoromethoxyphenylimidazole (**5**) yielded a mixture of two isomers which were separable by column chromatography. The preferred 2-substituted pyrimidine isomer, **7**, was reacted with a leucine derivative **8** to afford product **40**. The triazole analog **27** was prepared in an analogous manner except substituting 1,2,4-triazole for **5**.

The substituted phenylimidazoles (**10**) were prepared via two routes; refluxing bromoacetophenone (**9**) in formamide[4] or reacting benzaldehyde (**11**) with *p*-tolylsulfonyl isocyanide (TosMIC) followed by heating of intermediate **12** in methanolic ammonia (Scheme 2).[5]

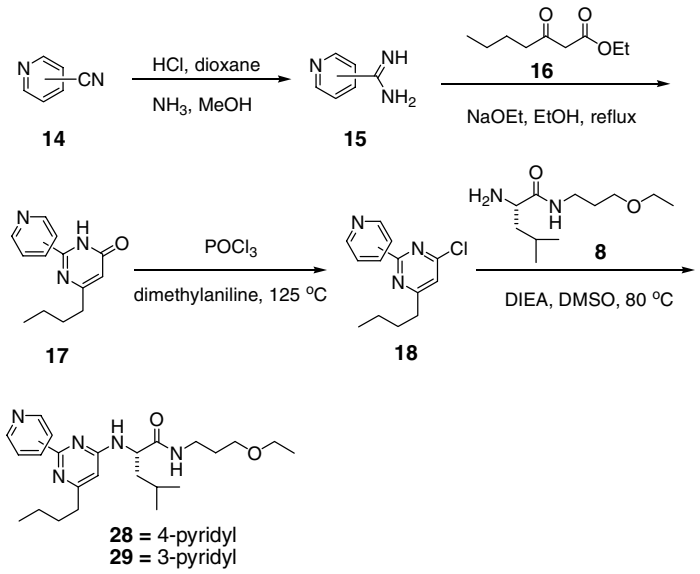


Scheme 2. Preparation of substituted phenylimidazole from bromoacetophenones or benzaldehyde.

Analogs **28** and **29**, the pyridylpyrimidines, were prepared by a different route (Scheme 3). Condensation of amidate **15** with β-ketoester **16** yielded a pyrimidinone **17**, which was converted to the chloropyrimidine **18** with phosphorus oxychloride. Displacement of the chloride with the leucine derivative **8** gave the final products (**28** and **29**).

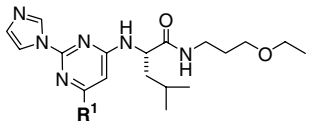
Although imidazolylpyrimidine **1** possessed good in vitro potency, it lacked stability with only 26% remaining after 30 min in the presence of rat liver microsomes (see Table 1) and had very poor bioavailability of much less than 1% in rat. The lack of microsomal stability was reflected with the relatively high clearance (1750 mL/h/kg) observed in vivo after iv dosing. The highly lipophilic *n*-octyl thioether moiety is prone to oxidation by the liver enzymes and likely to contribute to the instability of the compound. Thus, initial optimization efforts were focused on this domain (R^1) (Table 1). A twofold decrease in potency was observed when the thiol group was removed (**19**). Compounds with successive removal of two carbons from the chain, namely, the *n*-hexyl and *n*-butyl derivatives (**20** and **21**, respectively), led to a stepwise decrease in activity. With respect to microsomal stability, shortening of the chain length afforded more stable compounds. Rat liver microsomal stability was further enhanced via the addition of a branched alkyl group (*t*-butyl, compound **22**), however, this stability gain was offset by a lack of potency.

The *n*-butyl analog **21** showed a good balance between potency (1.7 μM) and microsomal stability (68% remaining after 30 min), and thus was used as the starting point for the next round of optimization—where the focus was to optimize the R^2 domain, the imidazole moiety (Table 2). A marked improvement in potency was observed with the addition of a methyl substituent to



Scheme 3. Preparation of pyridylpyrimidines.

Table 1. Modification of R^1 domain

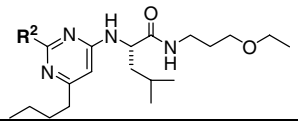


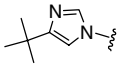
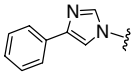
Compound	R^1	K_i[a] (nM)	RLM[b] (% rem)
1	(*n*-Octyl)-S-	60	26
19	*n*-Octyl-	120	36
20	*n*-Hexyl-	290	50
21	*n*-Bu-	1700	68
22	*t*-Bu-	6000	83

[a] Values are means of at least two experiments with standard deviation <±30%.

[b] Rat liver microsome stability was measured by incubation of compound (10 μM) in enzyme (300 nM) for 30 min at 37 °C. The % of compound remaining after 30 min was quantitated by a LC method. Values are means of two experiments, with a standard deviation of <±20%.

Table 2. Modification of R^2 domain



Compound	R^2	K_i[a] (nM)	RLM[b] (% rem)
21		1700	68
23		>10,000	27
24		90	25
25		85	13
26		100	2
27		>10,000	30
28		>5000	52
29		750	36

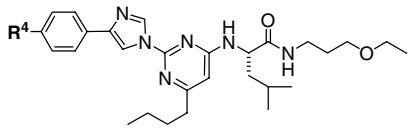
See Table 1 for footnote a and b.

the 4-position of the imidazole ring (**24**). Conversely, substitution at the 2-position (**23**) was not tolerated and resulted in a significant loss of potency. Bulkier groups, such as a *t*-butyl (**25**) and phenyl (**26**), were permitted in the 4-position of the imidazole and afforded compounds with potency similar to that of the methyl analog **24**.

Replacement of the imidazole ring with a triazole (**27**) or 4-pyridine (**28**) group provided inactive compounds. However, a twofold improvement in potency was seen for the 3-pyridyl analog **29**, indicating that the nitrogen atom placement within the heteroaromatic R^2 group may be important for potency. Although many of the R^2 modifications yielded compounds that were more potent than **21**, all exhibited lower microsomal stability.

Further optimization of compound **26**, the 4-phenyl imidazole analog, illustrated that changing the electronic nature of the aryl ring altered the potency and in vitro metabolic profile of the series (Table 3). In general, all modifications at the 4-position (termed R^4) yielded compounds with improved potency over **26** (R^4 = H).

Table 3. Substituted phenyl analogs



Compound	R^4	K_i[a] (nM)	RLM[b] (% rem)
26	H-	100	2
30	Me-	40	35
31	MeO-	42	30
32	HO-	32	65
33	F_3CO-	17	66
34	Cl-	27	54
35	F-	37	20
36	F_3C-	14	80
37	NC-	43	50
38	O_2N-	160	48
39	H_2N-	600	nd

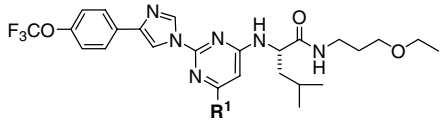
See Table 1 for footnote a and b.

Exceptions were the nitro (**38**) and amino (**39**) analogs. More importantly, these compounds showed substantial improvement in microsomal stability, with compounds **32**, **33**, **34**, and **36** having greater than 50% of the compound remaining after 30 min incubation with rat liver microsome.

With these more stable compounds in hand, the aliphatic R^1 group was re-visited with the aim to further reduce the lipophilicity of the moiety and was exemplified by the series based on compound **33** where carbons were sequentially removed from R^1 (Table 4). The calculated A log *P* reflects the overall change in lipophilicity brought in by reducing the size of the R^1 group.

The potency of the *n*-propyl analog (**40**) was comparable with the *n*-butyl (**33**) derivative, however, compounds with that of shorter R^1 groups are two- to threefold less active (compare **33** with **41** and **42**). Modification of this domain had little effect on microsomal stability. However, complete removal of the alkyl chain, R^1 = H, gave compound **43** that was significantly less active and less stable to rat liver microsomes.

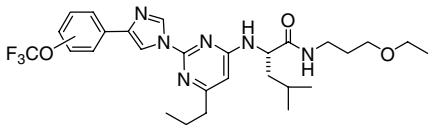
Table 4. R^1 homologs



Compound	R^1	K_i[a] (nM)	RLM[b] (% rem)	% *F* (rat)[c]	A log *P*
33	*n*-Bu-	17	66	7	6.74
40	*n*-Pr-	25	74	33	6.29
41	Et-	57	77	18	5.83
42	Me-	44	67	22	5.16
43	H-	200	5	4	4.88

See Table 1 for footnote a and b.
[c] Compound was dosed at 4 mg/kg iv and po.

Table 5. Positional isomers of the trifluoromethoxy substituent



Compound	Position of CF_3O-	K_i[a] (nM)	RLM[b] (% rem)	% *F* (rat)
40	*para*-	25	74	33
44	*meta*-	35	55	7
45	*ortho*-	50	13	5

See Table 1 for footnote a and b.

The potency of the *o*-, *m*-, and *p*-trifluoromethoxy positional isomers was similar (Table 5), however the ortho analog (**45**) was rapidly metabolized by the microsomes with only 13% remaining after 30 min, compared with 55% and 74% for the *meta* and *para* compounds (**44** and **40**, respectively).

Oral bioavailability data (% *F*) for the trifluoromethoxyphenyl series are shown in Tables 4 and 5. As a generalization in this series, compounds with good in vitro microsomal stability (>60% remaining after 30 min in

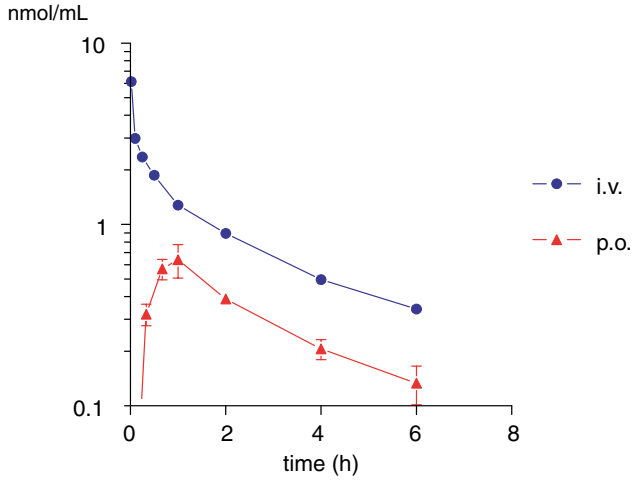


Figure 1. Pharmacokinetic profile of compound **40** in rats ($n = 3$) after dosing 4 μmol/kg iv and po.

Table 6. PK parameters of compound **40**

	Rat	Dog[a]
Dose (μmol/kg)		
iv	4	2
po	4	4
$t_{1/2}$ (h)	2.7 ± 0.05	1.1 ± 0.1
Cl (mL/h/kg)	590 ± 25	723 ± 3
V_{ss} (L/kg)	1.99 ± 0.06	2.79 ± 0.20
AUC_∞[b] (h nmol/mL)	2.43 ± 0.27	1.39 ± 0.70
C_{max}[b] (nmol/mL)	0.66 ± 0.12	0.30 ± 0.04
F (%)	33 ± 3	25 ± 12

[a] $n = 2$.
[b] po.

rat liver microsomes) showed reasonable oral bioavailability (% $F > 20$). Exception was seen with compounds **33** which illustrate that although subtle structural modification may not dramatically affect metabolic stability, they may profoundly affect oral bioavailability, as seen in the comparison of *n*-butyl and *n*-propyl analogs (**33** and **40**). It may be hypothesized that the addition of an extra carbon atom negatively affects the physicochemical properties of **33** thereby reducing drug absorption leading to the observed difference in oral bioavailability.

The pharmacokinetic (iv and po) profile in rats and relevant PK parameters (rat and dog) of compound **40** are shown in Figure 1 and Table 6, respectively.

Compound **1**, obtained from the CXCR2 chemokine receptor antagonist screening program, was optimized for potency and in vitro metabolic stability. Metabolism related to the bulky and lipophilic *n*-octyl thio group was addressed by replacing it with smaller aliphatic moieties (R^1). Optimization of the imidazole domain (R^2) by introduction of the 4-(4-substituted-phenyl)imidazole group yielded particularly potent molecules. Pharmacokinetic studies performed on the series highlighted the importance of in vitro microsomal stability, which in this series appears to be one of the key determining factors for good oral availability. However, subtle structural changes within a series, such as altering chain length, can drastically affect pharmacokinetic profiles while having little effect on potency or microsomal stability. Two compounds (**40** and **42**)[6] were identified with both good potency ($K_i < 50$ nM) and oral bioavailability (>20 % in rat).

Acknowledgments

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References and notes

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6. Analytical data for a representative compound **40** (HCl salt): ^{1}H NMR (CD_3OD) δ 9.90 (s, 1H), 8.75 (s, 1H), 8.10 (overlapping s and d, 2H), 7.60 (d, 2H), 6.65 (s, 1H), 4.65 (m, 1H), 3.40 (m overlapped with solvent, 6H), 2.75 (t, 2H), 1.85 (m, 7H), 1.10 (m, 12H); MS (ESI) *m/z* 563.3 $(M+H)^+$.