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Lead identification of 2-iminobenzimidazole antagonists of the chemokine receptor CXCR3

Martin E. Hayes,* Eric C. Breinlinger, Grier A. Wallace, Pintipa Grongsaard, Wenyan Miao,† Michael J. McPherson, Robert H. Stoffel, David W. Green‡ and Gregory P. Roth§

Abbott Bioresearch Center, Medicinal Chemistry, 381 Plantation Street, Worcester, MA 01605, USA

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Abstract—Modification of a 2-iminobenzimidazole series derived from an HTS hit resulted in compounds with improved in-vitro species selectivity. Incorporation of an 8-quinoline amide and conformational rigidification of an aliphatic tether furnished potent compounds suitable for further lead optimization.

Chemokines are *chemo*tactic cyto*kines* that mediate leukocyte migration and recruitment in response to pro-inflammatory cytokines[1,2] They bind to 7-transmembrane G-protein coupled receptors on the surface of T cells, macrophages, and mononuclear cells, and the expression of these receptors is correlated with immune diseases such as multiple sclerosis. The receptor CXCR3 is found primarily on T cells and is specific for the chemokine ligands MIG (monokine induced by γ-interferon; CXCL9), IP-10 (γ-interferon-inducible protein 10; CXCL10), and I-TAC (interferon-inducible T cell α-attractant; CXCL11). MIG, IP-10 and I-TAC are not known to be ligands for any other chemokine receptors besides CXCR3. Binding of any one of these ligands to CXCR3 activates signaling pathways that result in actin polymerization, cytoskeletal rearrangements, adhesion, and ultimately cell activation and chemotaxis.

CXCR3 has been of interest as a therapeutic target due to its role in T cell chemotaxis and the presence of $CXCR3^+$ cells and CXCL10 in lesions from multiple sclerosis patients.[3] Recent studies using CXCR3 (−/−) mice have demonstrated a critical role for CXCR3 and CXCL10 in regulating leukocyte effector functions such as IFN-γ production in an autoimmune disease model.[4] Several small molecule antagonists of CXCR3 have been reported including 4-*N*-aryl-[1,4]diazepine ureas,[5] 1-aryl-3-piperidin-4-yl-ureas,[6] aminotropane derivatives,[7] *N*-arylimidazoles,[8] and a quinazolinone antagonist, AMG-487, which has been progressed into clinical trials for both psoriasis and rheumatoid arthritis.[9]

In our screen for inhibitors of CXCR3, we identified a benzimidazole hit (1) with moderate molecular weight and low *c*Log *P*. Through modifications of the heterocycle and benzenoid ring, we were able to identify tractable SAR for the pharmacophore (see Fig. 1) and address

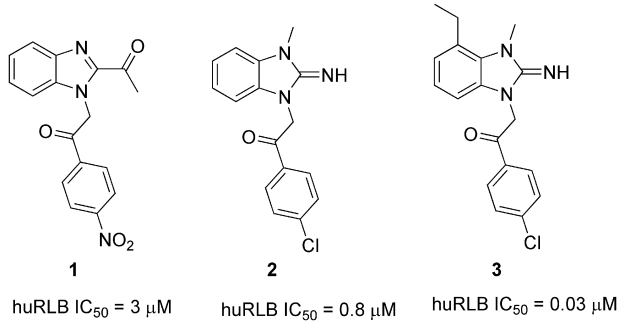

Figure 1. SAR identified during initial hit-to-lead efforts.

Keywords: Chemokine; Chemokine antagonist; CXCR3; IP-10; CXCL10; Multiple sclerosis; Benzimidazole; 2-Iminobenzimidazole.
* Corresponding author. Tel.: +1 508 688 8081; fax: +1 508 688 8100; e-mail: martin.hayes@abbott.com
† Present address: Wyeth Research, Cambridge, MA 02140, USA.
‡ Present address: Amgen Inc., Cambridge, MA 02139, USA.
§ Present address: Burnham Institute for Medical Research at Lake Nona, Orlando, FL 32819, USA.

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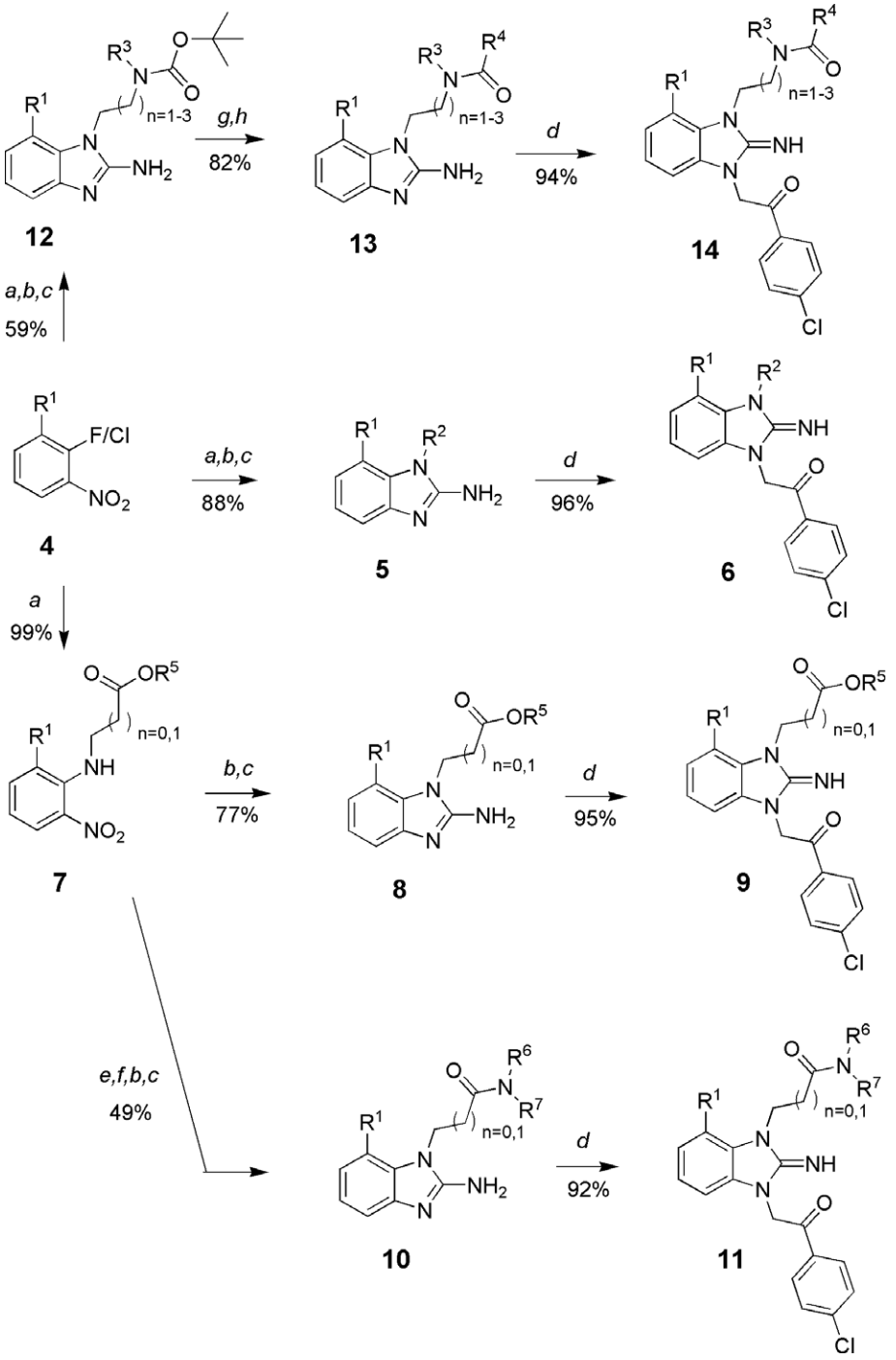
partial solubility associated with the core in order to obtain complete dose response values at the higher concentrations of the competition-binding assay.[10] Our interest then turned toward the impact of further substitution at the N-3 position of benzimidazoles such as **3**.

Substituents at the N-3 position of benzimidazoles such as **6** were introduced through nucleophilic aromatic substitution of an *o*-flouro- or an *o*-chloronitrobenzene (4) with an appropriate amine as shown in Scheme 1.[11] Reduction of the nitro group was effected with iron in protic acid[12] and formation of benzimidazoles such as **5** was achieved using an excess of BrCN in acetonitrile.[13] Alkylation with a bromoacetophenone under neutral conditions furnished benzimidazoles of type **6**.[14] Compounds such as **9** were prepared in the same manner using an amino ester nucleophile. To generate amide substrates of the type **11**, conversion of the ester to an



Scheme 1. Synthetic routes to N-3 substituted CXCR3 antagonists **6a**–**c**, **9a**, **11a**–**b**, and **14a**–**n**. Yields given in the scheme are for compounds **6a**, **9a**, **11b**, and **14f**. Reagents and conditions: (a) R^2NH_2, EtOH, reflux, 16 h; (b) Pd/C, NH_4CO_2H, EtOH, rt, 16 h; or Fe, AcOH, rt, 16–48 h; (c) BrCN, Na_2CO_3, MeCN, rt, 8–16 h; (d) $ArC(O)CH_2Br$, DMF, rt, 4–12 h; (e) NaOH, EtOH/H_2O (2:1), rt, 15 h; (f) $NH_2R^6R^7$, HOBt, DCC, THF, rt, 15 h; (g) TFA, CH_2Cl_2, rt, 2 h; (h) $ClCOR^4$, TEA, CH_2Cl_2, rt, 2 h.

amide moiety prior to benzimidazole formation was necessary in order to avoid a competing intramolecular reaction. For the preparation of reversed amides such as **13**, a mono-Boc protected diamine was employed in the aromatic substitution step. We reasoned that a Boc protecting group would be stable to the subsequent reduction and cyanogen bromide condensation steps in order to facilitate a late-stage diversification strategy. Several analogs of type **14**, derived from a variety of mono-Boc protected diamines were prepared in this manner.

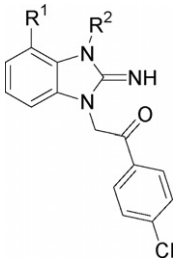
Compounds were initially evaluated for their ability to inhibit [^{125}I]-labeled CXCL10 binding to membranes of CHO cells stably expressing human CXCR3.[15] Functional antagonism was also measured in CHO cells using a FLIPR-based calcium mobilization assay.[16]

Our earlier work on the benzimidazole series identified small aliphatic substituents at the C-4 position to be an important element of the pharmacophore.[10] However, we chose to examine substitution at the N-3 position with less potent analogs devoid of this C-4 substitution in order to better gauge the impact of modifications. Initial replacement of the *N*-3 methyl with increasingly larger aliphatic groups showed no significant impact on the binding potency (entries 6a–c), as shown in Table 1. It is notable, though, that the receptor was tolerant of groups with increasing steric demand at this position. We next examined substitution at N-3 with more functionalized groups in order to identify an additional point of interaction of the receptor–ligand complex (entries 9a, 11a, 11b, 14a–d). While incorporation of increasingly larger, tethered-amides provided compounds that were essentially equipotent with **2** in the binding assay, the functional antagonism, as measured by a FLIPR assay, showed notable improvement with the incorporation of an extended amide group (compare entries 14c and 14d).

The improvement in functional activity with incorporation of *N*-benzylamides came with the addition of considerable molecular weight. We hoped to offset this substantial weight increase through optimization of binding activity associated with the distal amide moiety. We quickly crossed over the newly identified *N*-benzylamides onto our core with a chloro substituent at the C-4 position (entries 14e–g). We reasoned that a chloro group could provide an increase in potency (compare entries 14f and 14h) through potential interaction with a putative hydrophobic pocket of the receptor while avoiding potential metabolic liabilities that could be associated with small aliphatic groups such as methyl or ethyl. Variation of the tether identified a three-atom chain as optimal for the placement of the amide (entry 14f). We then executed a focused library of suitably diverse aryl, heteroaryl, and aliphatic amides to probe for a more optimal amide substituent. Several aryl groups with heteroatoms at the ortho position were identified as potent, functional antagonists at the human receptor, as shown in Table 2.

We were also interested in screening for potency at the mouse receptor since species differentiation is commonly observed with small molecule inhibitors of chemokines[17] and one of the early milestones for the project included in-vivo efficacy in a rodent model of multiple sclerosis. Compound **14n** stood out

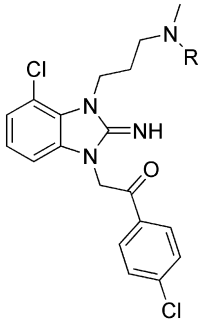
Table 1. SAR of N-3 substituted analogs

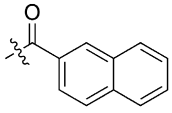
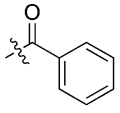
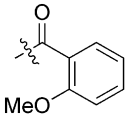
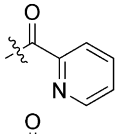
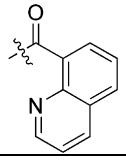


Entry	R^1	R^2	huRLB IC_{50}[a] (μM)	huFLIPR IC_{50}[a] (μM)	muRLB IC_{50}[a] (μM)
6a	H	Me	0.7	9	
6b	H	Et	0.7	3.5	
6c	H	Propyl	1.1	—	
9a	H	$CH_2CH_2CO_2Et$	1.2	2.5	
11a	H	$CH_2C(O)N(Me)CH_2Ph$	2.2	—	
11b	H	$CH_2CH_2C(O)N(Me)CH_2Ph$	0.4	0.2	
14a	H	$CH_2CH_2N(Me)C(O)Me$	1.3	3.4	
14b	H	$CH_2CH_2N(Me)C(O)C_6H_{11}$	0.5	0.9	
14c	H	$CH_2CH_2N(Me)C(O)Ph$	0.3	2.3	
14d	H	$CH_2CH_2N(Me)C(O)CH_2Ph$	0.4	0.8	
14e	Cl	$CH_2CH_2N(Me)C(O)CH_2Ph$	0.4	0.3	1.5
14f	Cl	$CH_2(CH_2)_2N(Me)C(O)CH_2Ph$	0.3	0.07	1.4
14g	Cl	$CH_2(CH_2)_3N(Me)C(O)CH_2Ph$	0.4	0.4	
14h	Et	$CH_2(CH_2)_2N(Me)C(O)CH_2Ph$	0.2	0.1	1.8

[a] IC_{50} values are an average of two runs.

Table 2. Library of tethered amides at N-3 position



Compound	R	huRLB IC_{50}[a] (μM)	huFLIPR IC_{50}[a] (μM)	muRLB IC_{50}[a] (μM)	MLM $T_{1/2}$ (min)
14i		0.6	0.4		
14J		0.015	0.04	0.5	9.5
14k		0.3	0.02	0.8	
14l		0.05	0.02	0.15	5.4
14m		0.03	0.02	0.2	
14n		0.02	0.02	0.05	15

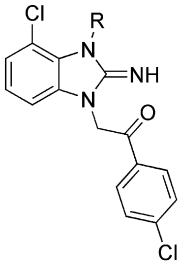
[a] IC_{50} values are an average of two runs.

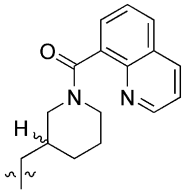
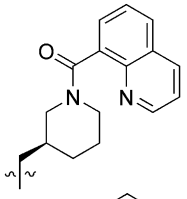
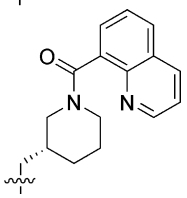
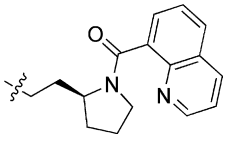
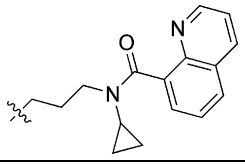
amongst the various amide-tethered compounds for its potency at both the human and murine receptors (compare entries 14n with 14l and 14m). However, this compound, as well as several amide-tethered substrates exhibited rapid transformation upon exposure to mouse liver microsomes (entries 14j, 14l, 14n). Mass spectral analysis of the microsome homogenates (data not shown) indicated that *N*-demethylation was the primary metabolic event in-vitro. To address this potential liability, we examined the impact of varying the *N*-methyl substituent. However, incorporation of larger aliphatic groups, such as the *N*-cyclopropyl moiety (Table 3, entry 14s), provided compounds that maintained potency at the human receptor but were less potent at the mouse receptor.

We considered another strategy to address the potential metabolic liabilities of an *N*-methyl amide tether. Constraining the *n*-propyl linker into a ring would provide a blocking group to minimize N-dealkylation while also reducing the number of rotatable bonds to enhance the drug-like properties of the molecule.[18] Replacement of the aliphatic tether with a piperidine ring, as shown in Table 3, was well-tolerated in-vitro showing no substantial impact on functional activity or binding potency across species (entry 14o). Each enantiomer was independently prepared (entries 14p–q) and no discernable preference for either stereoisomer was observed. Additional ring-containing tethers were explored and most were well tolerated with a 2-pyrrolidine amide (entry 14r) demonstrating excellent potency and functional activity across species. However, when compound **14r** was exposed to mouse liver microsomes, rapid modification ($T_{1/2}$ = 7.5 min) to an unidentified metabolite was observed.

In summary, through systematic modification of the N-3 position of compound **3**, we identified an *n*-propyl tethered amide containing an ortho-heteroatom motif, as exemplified by an 8-quinoline amide, as a key element of our pharmacophore. This group imparts substantial binding potency at both the human and mouse receptors. Through modification of the alkyl tether we also identified a number of cyclic amides

Table 3. Alternate tethers of 8-quinoline amide analogs



Compound	R	huRLB IC_{50}[a] (μM)	huFLIPR IC_{50}[a] (μM)	muRLB IC_{50}[a] (μM)
14o		0.015	0.02	0.08
14p		0.015	0.02	0.06
14q		0.016	0.02	0.07
14r		0.008	0.02	0.04
14s		0.02	0.01	0.10

[a] IC_{50} values are an average of two runs.

with excellent potency and functional activity that represent good candidates for further lead optimization.

Acknowledgments

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15. Radioligand binding assays were performed in CHO cells expressing human or murine CXCR3. All compounds were dissolved in DMSO and assays run at a final DMSO concentration of 1% (v/v). [^{125}I]-labeled CXCL10 was purchased from PerkinElmer and used at 50 pM per assay.
16. Calcium flux assays were performed in CHO cells expressing human CXCR3 and the $G\alpha_{16}$ coupling protein using a FLIPR instrument (Molecular Devices). All compounds were dissolved in DMSO and assays run at a final DMSO concentration of 1% (v/v). Human CXCL10 was purchased from Peprotech and used at a final assay concentration of 30 nM.
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