Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 1573–1576
Bioorganic & Medicinal Chemistry Letters

Discovery of small molecule benzimidazole antagonists of the chemokine receptor CXCR3

Martin E. Hayes,* Grier A. Wallace, Pintipa Grongsaard, Agnieszka Bischoff, Dawn M. George, Wenyan Miao,† Michael J. McPherson, Robert H. Stoffel, David W. Green‡ and Gregory P. Roth§

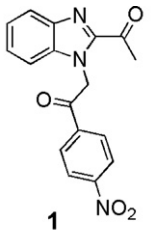
Abbott Bioresearch Center, 381 Plantation Street, Worcester, MA 01605, USA

Received 17 December 2007; revised 18 January 2008; accepted 18 January 2008
Available online 26 January 2008

Abstract—High-throughput screening identified a low molecular weight antagonist of CXCR3 displaying micromolar activity in a membrane filtration-binding assay. Systematic modification of the benzimidazole core and tethered acetophenone moiety established tractable SAR of analogs with improved physicochemical properties and sub-micromolar activity across both human and murine receptors.
© 2008 Elsevier Ltd. All rights reserved.

Chemokines are a subfamily of *chemo*tactic cyto*kines* that bind to cell surface G-protein coupled receptors (GPCRs) and are responsible for modulating leukocyte migration.[1] A number of these receptors and their endogenous ligands are thought to play a key role as pro-inflammatory mediators of autoimmune diseases[2] such as multiple sclerosis[3] and rheumatoid arthritis.[4] The chemokine IP-10 (CXCL10) and its receptor, CXCR3, are highly expressed within the CNS at sites of demyelination in multiple sclerosis patients.[5] Studies using neutralizing antibodies for CXCL10 have demonstrated efficacy at preventing disease onset in a rodent EAE model of multiple sclerosis.[6] Therefore, small molecule antagonists of CXCR3 would be of interest as a potential therapy for the treatment of autoimmune disorders such as multiple sclerosis. Several small molecule antagonists of CXCR3 have been reported including 4-*N*-aryl-1,4-diazepine ureas,[7] 1-aryl-3-piperidin-4-yl-ureas, and 2-amino(4-piperidinyl)azoles.[8] Another small molecule, AMG-487, has been progressed into clinical trials for both psoriasis and rheumatoid arthritis.[9]

A high-throughput screen of the Abbott corporate compound collection identified **1** as a moderately potent (IC_{50} = 3 μM), functional antagonist of CXCR3. The moderate molecular weight (323) and *c*Log *P* (2.7) of the compound made it an attractive starting point for our hit-to-lead effort (Fig. 1). Three different synthetic approaches were utilized for the synthesis of analogs as summarized in Scheme 1. 2-Acylbenzimidazoles such as **4** and 2-alkylbenzimidazoles such as **9** were prepared by condensation of aryldiamines of the type **2** with an appropriate carboxylic acid at elevated temperatures followed by oxidation to prepare compounds of the type **3**.[10] Alkylation of the resulting benzimidazoles was



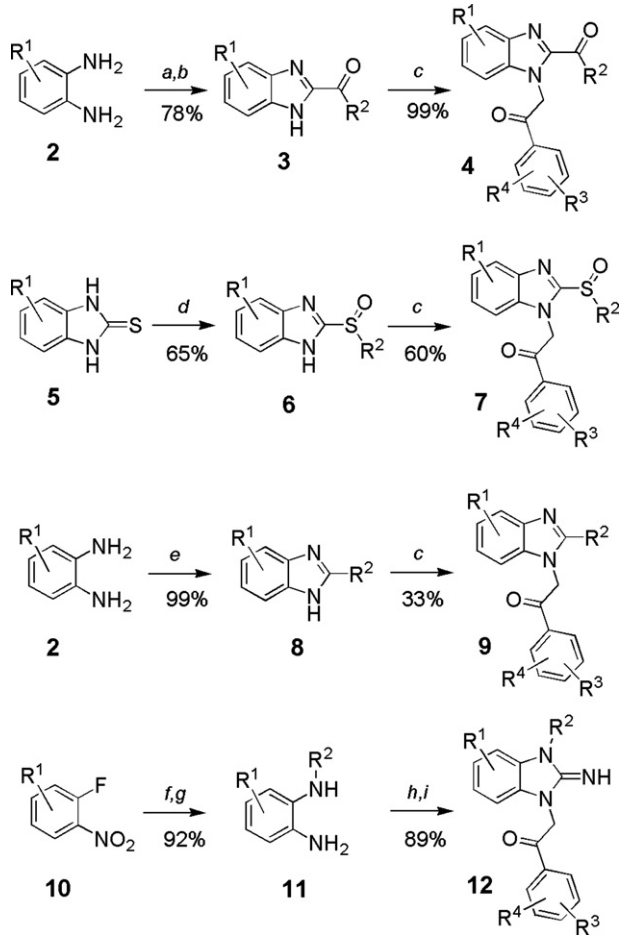
1

cLogP = 2.7 MW = 323.30 huRLB IC_{50} = 3 μM

Figure 1. CXCR3 antagonist hit from HTS.

Keywords: Chemokine; Chemokine antagonist; CXCR3; IP-10; CXCL10; Multiple sclerosis; Benzimidazole; 2-Iminobenzimidazole; Partial solubility.
* 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.

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.01.074



Scheme 1. Synthesis of 2-substituted benzimidazoles **4a**–**j**, **7a**, **9a**, **b9**, and **12a**–**q**. Yields given in the scheme are for compounds **4b**, **7a**, **9a**, and **12e**. Reagents and conditions: (a) $R^2CH(OH)CO_2H$, 4 N HCl, 90 °C, 16 h; (b) Dess–Martin Periodinane, CH_2Cl_2, rt, 16 h; (c) $ArC(O)CH_2Br$, K_2CO_3, Acetone, rt, 4 h; (d) R^2I, K_2CO_3, MeCN, rt, 16 h, then *m*CPBA, CH_2Cl_2, rt, 16 h; (e) R^2CO_2H, 4 N HCl, 90 °C, 16 h; (f) R^2NH_2, EtOH, reflux, 16 h; (g) Pd/C, NH_4CO_2H, EtOH, rt, 16 h; or Fe, HCl, EtOH, reflux, 16–48 h; (h) BrCN, MeCN, rt, 8–16 h; (i) $ArC(O)CH_2Br$, DMF, rt, 4–12 h.

effected by treatment with an α-bromoacetophenone in the presence of potassium carbonate.[11] Sulfoxide analogs **7** were prepared from the corresponding benzothiourea via S-alkylation and oxidation.[12,13] Preparation of 2-iminobenzimidazoles **12** was accomplished by utilizing an addition–elimination protocol. Thus, treatment of an *o*-fluoronitroarene with an appropriately substituted amine followed by reduction of the nitro group using catalytic hydrogenation[14] or iron in protic acid[15] and subsequent reaction of the resulting diamines **11** with cyanogen bromide provided 2-iminobenzimidazoles.[16] Further elaboration by alkylation with an α-bromoacetophenone gave the desired product **12**.

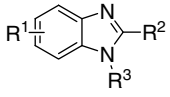
Compounds were initially evaluated for their ability to inhibit [^{125}I]-labeled CXCL10 binding to membranes of CHO cells stably expressing human CXCR3.[17] Functional antagonism was also measured in CHO cells using a FLIPR-based calcium mobilization assay.[18]

We initially examined the impact of modifications to the acetophenone moiety as shown in Table 1. Replacement of the 4-nitro substituent with isosteres such as the cyano group (entry 4a) was not tolerated but halogen groups (entries 4b and 4c) provided binding potencies similar to that of the lead. Removing the para-substituent also resulted in compounds with significantly reduced potency (entry 4d). Substitution at the C-5 and C-6 position of the benzimidazole core with groups such as methoxy (entries 4f and 4g) and phenyl (entries 4h and 4i) resulted in weakly potent compounds while substitution at C-4 with methoxy (entry 4e; IC_{50} = 3 μM) showed no change in the binding assay. Several modifications of the 2-acyl group, including replacement of the carbonyl with a sulfoxide (entry 7a) and reduction of the carbonyl to the corresponding carbinol (entry 9a), resulted in compounds having reduced potency.

Analogs in the 2-acyl series, as well as many analogs containing acyl isosteres, were poorly soluble in aqueous buffer at the higher concentrations of the assay. This resulted in an incomplete dose response curve and an apparent partial antagonism that complicated SAR interpretation for these compounds. However, substitution of the 2-acyl for an imino group provided compounds with improved solubility[19] which allowed for complete dose response curves that typically provided >80% inhibition relative to the endogenous ligand (CXCL10). All future analogs incorporated this 2-imino group because of both the improved potency and increased solubility it afforded. Extended substitution on this 2-imino group invariably resulted in a significant reduction in potency as shown in Table 2 (entries 12a–c). Only small aliphatic substituents were tolerated with the 2-methylimino analog (entry 12c) being equipotent to the unsubstituted 2-imino compound (entry 12d).

Having successfully improved solubility by incorporating the 2-imino substituent on the core, we next investigated substitution on the acetophenone group. As shown in Table 2, both bromine and chlorine were suit-

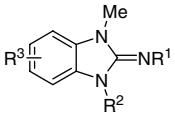
Table 1. Binding potency of analogs



Entry	R^1	R^2	R^3	huRLB IC_{50}[a] (μM)
4a	H	C(O)Me	$CH_2C(O)Ph(4\text{-}CN)$	>100
4b	H	C(O)Me	$CH_2C(O)Ph(4\text{-}Br)$	2
4c	H	C(O)Me	$CH_2C(O)Ph(4\text{-}Cl)$	8
4d	H	C(O)Me	$CH_2C(O)Ph$	>100
4e	4-OMe	C(O)Me	$CH_2C(O)Ph(4\text{-}Br)$	3
4f	5-OMe	C(O)Me	$CH_2C(O)Ph(4\text{-}Br)$	>50
4g	6-OMe	C(O)Me	$CH_2C(O)Ph(4\text{-}Br)$	>50
4h	5-Ph	C(O)Me	$CH_2C(O)Ph(4\text{-}Br)$	>50
4i	6-Ph	C(O)Me	$CH_2C(O)Ph(4\text{-}Br)$	>50
7a	H	S(O)Me	$CH_2C(O)Ph(4\text{-}NO_2)$	11
9a	H	CH(OH)Me	$CH_2C(O)Ph(4\text{-}NO_2)$	20

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

Table 2. Binding and functional antagonism of 2-iminobenzimidazole analogs



Entry	R^1	R^2	R^3	huRLB IC_{50}[a] (μM)	huFLIPR IC_{50}[a] (μM)
12a	C(O)Me	$CH_2C(O)Ph(4\text{-}NO_2)$	H	50	
12b	CH_2CH_2OMe	$CH_2C(O)Ph(4\text{-}Br)$	H	1	
12c	CH_3	$CH_2C(O)Ph(4\text{-}Cl)$	H	0.8	
12d	H	$CH_2C(O)Ph(4\text{-}Cl)$	H	0.8	9
12e	H	$CH_2C(O)Ph(4\text{-}Br)$	H	0.8	
12f	H	$CH_2C(O)Ph(3,4\text{-}diCl)$	H	4	
12g	H	$CH_2C(O)Ph(3\text{-}Cl)$	H	16	
12h	H	$CH_2C(O)Ph(2\text{-}Cl)$	H	22	
12i	H	$CH_2S(O)Ph(4\text{-}Cl)$	H	3	
12j	H	$CH_2CH_2Ph(4\text{-}Br)$	H	11	
12k	H	$CH_2CH_2CH_2C(O)Ph(4\text{-}Br)$	H	6	
12l	H	$CH_2CH(OH)Ph(4\text{-}Br)$	H	40	
12m	H	$CH_2C(O)Ph(4\text{-}Cl)$	6-Me	8	
12n	H	$CH_2C(O)Ph(4\text{-}Cl)$	5-Me	1	5
12o	H	$CH_2C(O)Ph(4\text{-}Cl)$	4-Me	0.1	0.08
12p	H	$CH_2C(O)Ph(4\text{-}Cl)$	4-Et	0.03	0.07
12q	H	$CH_2C(O)Ph(4\text{-}Cl)$	4-*n*Pr	0.5	0.4
12r	H	$CH_2C(O)Ph(4\text{-}Cl)$	4-Cl	0.3	
12s	H	$CH_2C(O)Ph(4\text{-}Cl)$	$4\text{-}CF_3$	0.7	
12t	H	$CH_2C(O)Ph(4\text{-}Br)$	4-OMe	0.8	

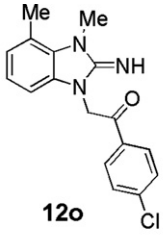
[a] IC_{50} values are an average of two runs.

able replacements for the 4-nitro group (entries 12d and 12e). Substitution at the 2- or 3-positions resulted in reduced potency (entries 12f–h). Isosteric replacement of the ketone with a sulfoxide (entry 12i) also resulted in a notable decrease in potency. Efforts to reduce the electrophilic ketone to a carbinol (entry 12l) or methylene (entry 12j) resulted in a loss of potency. Chain extension of the acetophenone up to two atoms (entry 12k) indicated that one methylene unit was preferred.

We next re-evaluated the impact of substitution on the benzimidazole core as shown in Table 2. Substitution with a methyl group at the C-6 position (entry 12m) was not tolerated while compounds possessing substitution at C-5 (entry 12n) were nearly equipotent with compound **12d**. A substantial boost in potency was observed with methyl substitution at the C-4 position (entry 12o). Other small groups also resulted in improved potency (entry 12r) including the ethyl-substituted compound **12p** that showed a 25-fold increase in potency relative to **12d**. In addition, an increase in functional antagonism (FLIPR) was observed with compounds incorporating small aliphatic groups at the C-4 position (compare entry 12o with 12d). Larger groups, such as propyl (entry 12q), and more polar substituents (entries 12s and 12t) did not show a significant improvement in potency suggesting that substituents at the C-4 position may potentially be binding within a small lipophilic pocket of the receptor.

Compound **12o** was further profiled for its pharmacokinetic properties in mouse to establish a benchmark for the chemotype (Fig. 2). Compound **12o** exhibited good bioavailability and half-life upon oral administration[20] along with limited stability in rat liver microsomes (49% parent remaining after 30 min) and modest protein binding (85%) in rat plasma. Compound **12o** also demonstrated consistent activity across species in the binding assay with good alignment in the functional assay (FLIPR) as shown in Table 2.

In summary, from an internal screen of our corporate compound collection we identified a low micromolar antagonist of CXCR3. Switching to a 2-iminobenzimidazole core resolved an apparent partial antagonism due to limited solubility and also afforded compounds with improved potency that demonstrated tractable SAR. Incorporation of small, non-polar groups at the C-4 position of the core resulted in notable potency gains and improved functional antagonism along with



12o

F (%)	$T_{1/2}$ (h)	Cl (L/h/Kg)	IC_{50} mu/IC_{50} hu
57	4.9	5.7	2

Figure 2. Pharmacokinetic properties and species selectivity of compound **12o**.

favorable pharmacokinetic properties to support further optimization efforts.

Acknowledgments

The authors acknowledge Elizabeth Alder and Lorraine McGuinness for generating radio-ligand binding and FLIPR assay data, Linda E. Chovan and Candace L. Black-Schaeffer for generating HT-ADME reports, and Brian Bettencourt for acquiring in-vivo pharmacokinetics data.

References and notes

1. D'Ambrosio, D.; Panina-Bordignon, P.; Sigigaglia, F. *J. Immunol. Methods* **2003**, *273*, 3.
2. Godessart, N. *Ann. N.Y. Acad. Sci* **2005**, *1051*, 647.
3. Sorensen, T. L.; Tani, M.; Jensen, J.; Pierce, V.; Lucchinetti, C.; Folcik, V. A.; Qin, S.; Rottman, J.; Selleberjerg, F.; Strieter, R. M.; Frederiksen, J. L.; Ransohoff, R. M. *J. Clin. Invest.* **1999**, *103*, 807.
4. Qin, S.; Rottman, J. B.; Myers, P.; Kassam, N.; Weinblatt, M.; Loetscher, M.; Koch, A. E.; Moser, B.; Mackay, C. R. *J. Clin. Invest.* **1998**, *101*, 746.
5. Simpson, J. E.; Newcombe, J.; Cuzner, M. L.; Woodroofe, M. N. *Neuropathol. Appl. Neurobiol.* **2000**, *26*, 133.
6. (a) Tsunoda, I.; Lane, T. E.; Blackett, J.; Fujinami, R. S. *Multiple Sclerosis* **2004**, *10*, 26; (b) Liu, L.; Callahan, M. K.; Huang, D.; Ransohoff, R. M. *Curr. Topics Dev. Biol.* **2005**, *68*, 149.
7. Cole, A. G.; Stroke, I. L.; Brescia, M-R.; Simhadri, S.; Zhang, J. J.; Hussain, Z.; Snider, M.; Haskell, C.; Ribeiro, S.; Appell, K. C.; Henderson, I.; Webb, M. L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 200.
8. (a) Allen, D. R.; Bolt, A.; Chapman, G. A.; Knight, R. L.; Meissner, J. W. G.; Owen, D. A.; Watson, R. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 697; (b) Watson, R. J.; Allen, D. R.; Birch, H. L.; Chapman, G. A.; Hannah, D. R.; Knight, R. L.; Meissner, J. W. G.; Owen, D. A.; Thomas, E. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6806; (c) Watson, R. J.; Allen, D. R.; Birch, H. L.; Chapman, G. A.; Galvin, F. C.; Jopling, L. A.; Knight, R. L.; Meier, D.; Oliver, K.; Meissner, J. W. G.; Owen, D. A.; Thomas, E. J.; Tremayne, N.; Williams, S. C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 147; (d) Knight, R. L.; Allen, D. R.; Birch, H. L.; Chapman, G. A.; Galvin, F. C.; Jopling, L. A.; Lock, C. J.; Meissner, J. W. G.; Owen, D. A.; Raphy, G.; Watson, R. J.; Williams, S. C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 629.
9. (a) Berry, K., et al. *Inflamm. Res.* **2004**, *Suppl. 3*, S197–S234 (abstract A089); (b) Johnson, M. G.; Li, A.; Liu, J.; Marcus, A. P.; Huang, A. X.; Medina, J. C. *Abstracts of Papers*, 231st National Meeting of the American Chemical Society, Atlanta, GA, March 26–30, 2006; (c) Johnson, M.; Li, A.-R.; Liu, J.; Fu, Z.; Zhu, L.; Miao, S.; Wang, X.; Xu, Q.; Huang, A.; Matcus, A.; Xu, F.; Ebsworth, K.; Sablan, E.; Danao, J.; Kumer, J.; Dairaghi, D.; Lawrence, C.; Sullivan, T.; Tonn, G.; Schall, T.; Collins, T.; Medina, J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3339; (d) Du, X.; Chen, X.; Mihalic, J. T.; Deignan, J.; Duquette, J.; Li, A.-R.; Lemon, B.; Ma, J.; Miao, S.; Ebsworth, K.; Sullivan, T. J.; Tonn, G.; Collins, T. L.; Medina, J. C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 608; (e) Li, A.-R.; Johnson, M. G.; Liu, J.; Chen, X.; Du, X.; Mihalic, J. T.; Deignan, J.; Gustin, D. J.; Duquette, J.; Fu, Z.; Zhu, L.; Marcus, A. P.; Bergeron, P.; McGee, L. R.; Danao, J.; Lemon, B.; Carabeo, T.; Sullivan, T.; Ma, J.; Tang, L.; Tonn, G.; Collins, T. L.; Medina, J. C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 688.
10. Ramaiah, K.; Grossert, J. S.; Hooper, D. L.; Dubey, P. K.; Ramanatham, J. *J. Indian Chem. Soc.* **1999**, *76*, 140.
11. Demirayak, S.; Mohsen, U. A.; Karaburun, A. C. *Eur. J. Med. Chem. Chim. Ther.* **2002**, *37*, 255.
12. Gardiner, J. M.; Loyns, C. R. *Tetrahedron* **1995**, *51*, 11515.
13. Graber, D. R.; Morge, R. A.; Sih, J. C. *J. Org. Chem.* **1987**, *52*, 4620.
14. Li, Q.; Li, T.; Woods, K. W.; Gu, W.; Cohen, J.; Stoll, V. S.; Galicia, T.; Hutchins, C.; Frost, D.; Rosenberg, S. H.; Sham, H. L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2918.
15. Shepherd, T.; Smith, D. M. *J. Chem. Soc., Perkin Trans. 1* **1987**, 501.
16. Valdez, J.; Cedillo, R.; Hernandez-Campos, A.; Yepez, L.; Hernandez-Luis, F.; Navarrete-Vazquez, G.; Tapia, A.; Cortes, R.; Hernandez, M.; Castillo, R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2221.
17. 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 Perkin-Elmer and used at 50 pM per assay.
18. 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.
19. For example, equilibrium solubility as measured by CLND detection of a 100-mM aqueous solution of compound **1** is 4.5 μM as compared with 84 μM for compound **12d** and 79 μM for compound **12o**.
20. Female SJL mice were obtained from Jackson Labs and compound **12o** was dosed in a vehicle of D5W with 5% Ethanol, 50% PEG 200, and 1 equivalent of 2 M HCl:10 mpk (po) by gavage and 5 mpk (iv) as a slow bolus in a tail vein.