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Synthesis and structure–activity relationship of benzetimide derivatives as human CXCR3 antagonists

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ABSTRACT

The synthesis and evaluation of benzetimide derivatives showing potent CXCR3 antagonism are described. Optimization of the screening hits led to the identification of more potent CXCR3 antagonists devoid of anti-cholinergic activity and identification of the key pharmacophore moieties of the series.

Cell migration is largely mediated by the interaction of some 50 secreted proteins, called chemokines, and 20 chemokine G protein-coupled receptors.[1] They have been divided into 4 families according to the number and spacing of their conserved N-terminal cysteine residues (CC, CXC, CX_3C, and XC). Chemokines and their receptors are also classified as constitutively expressed (homeostatic) or inducible/inflammatory (to control cell recruitment to sites of infection and inflammation). Human (h)CXCR3 is predominantly expressed on activated T helper 1(Th1) cells and activated by the interferon-inducible chemokines MIG (CXCL9), IP10 (CXCL10), and ITAC (CXCL11).[2] Its function is mediated through $G_{\alpha i}$ protein binding, leading to inhibition of cAMP formation and enhanced calcium mobilization, actin polymerization, and chemotaxis.[3,4] Upregulation of hCXCR3 and its ligands, together with increased levels of disease-causing activated T cells in inflamed lesions, has been demonstrated in several chronic inflammatory diseases including organ transplant rejection,[5] multiple sclerosis,[6] colitis,[7] insulitis,[8] chronic obstructive pulmonary disease,[9] allergic dermatitis,[10] and rheumatoid arthritis.[11] Taken together, these data indicate that inhibition of hCXCR3 activation provides a potential therapeutic intervention in a number of major Th1 cell-mediated inflammatory disorders. Recently, a number of experimental compounds directed to the CXCR3 target were reported.[12] Herein we report the identification of a novel series of hCXCR3 antagonists with promising activity and drug-like properties.

Through screening of some 256,000 compounds for their inhibitory activity on cAMP production in ITAC-stimulated hCXCR3-transfected CHO cells,[13] a series of 3-disubstituted piperidine-2,6-diones analogs of benzetimide (**3**) as novel hCXCR3 antagonists were identified as shown in Fig. 1. The activity of the most potent hit compound (**1**) was confirmed by assessing its ability to block the ITAC-activated GTPγS binding to hCXCR3-transfected CHO cell membranes,[14] and compared to AMG 487 (**2**), the most advanced hCXCR3 antagonist at the time.[15]

As shown in Table 1, compound **1** inhibited the hCXCR3-mediated GTPγS binding with an IC_{50}-value of 0.8 μM, comparable to that of AMG 487 (IC_{50} = 0.3 μM).

The realization that **1** structurally resembles the muscarinic receptor antagonist benzetimide (**3**), prompted us to assess its potential anti-cholinergic activity. Confirming earlier data,[17] the anti-

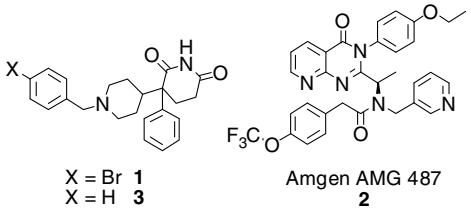

Figure 1. CXCR3 antagonists.

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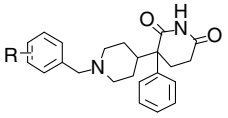
Table 1
hCXCR3 antagonism and muscarinic binding data of compounds **1**–**3** and their enantiomers

Compound	Chirality	hCXCR3 binding IC_{50}[a] (μM)	Muscarinic receptor binding IC_{50}[b] (μM)		
			M1	M2	M3
1	±	0.78			
1a	–	0.61	9.6	>10	>10
1b	+	1.33	<0.001	<0.001	<0.001
2	–	0.33	n.d.	n.d.	n.d.
3	±	>10			
3a	–	>10	>10	>10	>10
3b	+	>10	<0.001	<0.001	<0.001

[a] GTPγS binding assay.[14]
[b] Ref. 16 for assay.

Table 2
hCXCR3 antagonism: N-substitution on the 4-piperidine ring



Compound[a]	R	hCXCR3 binding IC_{50}[b] (μM)
1	4-Br	0.78
3	H	>10
6	4-Cl	0.83
7	4-F	4.57
8	4-CH3	2.40
9	4-OMe	4.27
10	2,4-Cl	3.16
11	3,4-Cl	1.26
12	3-F-4-Cl	0.34
13	3-CF_3-4-Cl	2.51

[a] Racemic compounds, except for the (–)-isomer of compound **12**.
[b] GTPγS binding assay.[14]

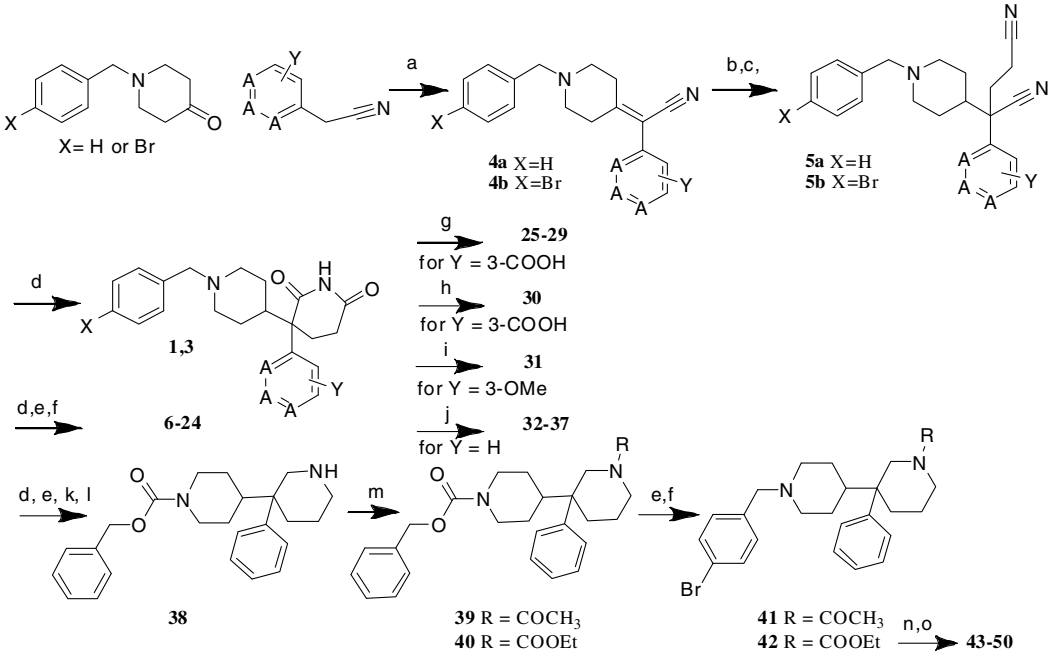
cholinergic activity of **3**, measured by blocking the binding of *N*-methylscopolamine to the human M1-3 muscarinic receptors, resides exclusively in its (+)-isomer, dexetimide (**3b**). By analogy, separation of **1** into its enantiomers **1a** and **1b** indicated that, although both stereoisomers produced comparable hCXCR3 antagonistic effects (Table 1), **1b** possessed nanomolar activity against the muscarinic receptors, whereas **1a** only showed a marginal activity toward the M1 receptor (IC_{50} = 9.6 μM). Moreover, submicromolar concentrations of **1a** also reduced the ITAC-induced migration of activated human blood T lymphocytes (IC_{50} = 0.69 μM),[18] and blocked the binding of [^{125}I]ITAC to stimulated human T lymphocytes (IC_{50} = 0.33 μM).[19] Additionally, when tested at 10 μM, **1a** had no effect (<25% inhibition) on the binding of CHO cells expressing human CCR1, CCR2, CCR4, CXCR1/2, and CX3CR1 with [^{125}I]-labeled MIP-1α, MCP-1, TARC, IL-8, and fractalkine, respectively.[20] Reassured by these observations, we sought to identify more potent and selective hCXCR3 antagonists.

Because of the pronounced difference in hCXCR3 binding potency between the para-bromo substituted benzyl-containing **1** (IC_{50} = 0.8 μM) and the unsubstituted benzyl analog **3**, we first explored the contribution of N-substitution of the 4-piperidine ring.

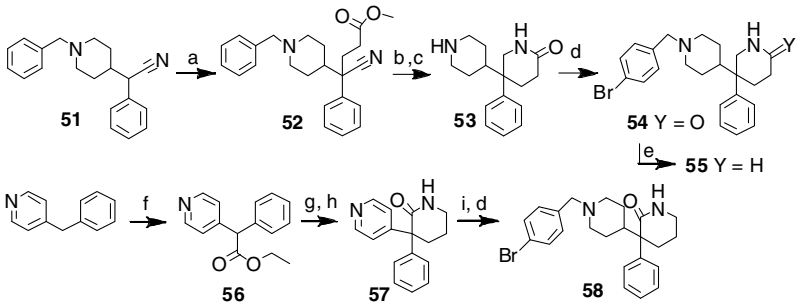
As shown in Table 2, the hCXCR3 antagonistic potency of **1** was found to be among the highest, and therefore we retained the *para*-bromobenzyl substituent and concentrated our efforts on the glutarimide portion of the molecule.

The synthesis of compounds **1**, **3–50** is depicted in Scheme 1.[21]

The build-up of the dexetimide analogs required the base-catalyzed condensation of the benzyl-piperidone with the appropriate arylated acetonitriles. The alkene was reduced either catalytically or with $NaBH_4$. The Michael addition with acrylonitrile promoted by Triton B was followed by the acid-mediated ring closure toward the glutarimide analogs **1**, **3**, and **6–24**. Compound **21** containing the 2-OMe-5-sulfonic acid group was obtained in low overall yield (1.6% starting from acrylonitrile addition step b) as a side product



Scheme 1. Reagents and conditions: (a) NaOMe, MeOH, reflux,18 h; (b) $NaBH_4$, iPOH, reflux or 10%Pd/C, thiophene, H_2, MeOH, rt; (c) acrylonitrile, Triton B, dry dioxane, 0 °C to rt, 18 h; (d) H_2SO_4/HOAc (1:4 v/v), 165 °C, 3–4 h, 10–40% for four steps; (e) 10% Pd/C, H_2, MeOH, rt, >90%; (f) opt. substituted benzylhalide, triethylamine, DMF or CH_2Cl_2/MeOH 1:1, rt, 18 h, variable yields; (g) $SOCl_2$, CH_2Cl_2, rt, 4 days, then amines, rt; (h) $(PhO)_2P(O)N_3$, *t*-BuOH, 85 °C, 18 h then 10%TFA in CH_2Cl_2, rt, 18 h, 22% for two steps; (i) BBr_3, CH_2Cl_2, rt, 18 h, 38%; (j) R-X, K_2CO_3, DMF, 60–70 °C, variable yields; (k) CBzCl, TEA, DMF, rt, 18 h, 71%; (l) BH_3·THF 5 equiv, reflux, 6 h, 100%; (m) Ac_2O, THF, rt, 18 h, 35% or $ClCO_2Et$, triethylamine, CH_2Cl_2, rt, 18 h, 37%; (n) 48% HBr, reflux, 2 h, 70%; (o) **43**: *n*BuOCHO, 100 °C, 4 h, 33%; **44**: trifluoroacetic anhydride, THF, rt, 4 days, 37%; **45**, **46**: RCOCl, triethylamine, CH_2Cl_2, rt, 65–77%; **47**: $ClCH_2CONH_2$, triethylamine, CH_2Cl_2, rt, 18 h, 49%; **48**: PheNCO, CH_2Cl_2, rt, 20 h, 64%; **49**: Me_3SiNCO, dry dioxane, 90 °C, 20 h, 14%; **50**: $NH_2SO_2NH_2$, pyridine, 120 °C, 18 h, 16%.



Scheme 2. Reagents and conditions: (a) methylacrylate, NaOMe, xylene, 65 °C, 4–6 h, 92%; (b) 10% Pd/C, H_2, MeOH, rt; (c) Raney nickel, H_2, MeOH, rt, 56% for two steps; (d) *p*-Br benzylchloride, triethylamine, DMF, rt, 18 h, 40–45%; (e) BH_3·THF, reflux, 18 h, 94%; (f) diethylcarbonate, LDA, THF, −78 °C, 40 min to rt, 2 h; (g) acrylonitrile, Triton B, dry dioxane, rt,18 h; (h) Raney nickel, H_2, THF, rt, 25% for three steps; (i) Pd/C, H_2, MeOH, 75 °C, 80%.

during an attempt to synthesize the 2-methoxy substituted variant. Acid chloride activation of the acid **24** enabled the formation of amides **25–29**. The two-step Curtius rearrangement of acid **24** yielded the aniline **30**. BBr_3 mediated demethylation of **20** yielded the phenol **31**. Base-promoted alkylation of the imide **1** produced **32–37**. The borane reduction of the imide did not proceed on the benzyl-protected piperidine derivatives, but was quantitative on the CBz-protected intermediate leading to intermediate **38**. Further coupling and deprotection steps produced the required compounds **43–50**.

The lactam analogs **54**, **58** of the lead compounds were synthesized as described in Scheme 2.[21]

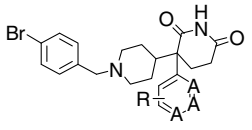
A Michael addition of methylacrylate on intermediate **51** was followed by the one-pot, two-step Pd-mediated debenzylation and the Raney nickel reduction of the nitrile to the amine, which spontaneously ring closed to lactam **53**. Coupling of *p*-bromobenzylchloride gave **54** and lactam reduction produced **55**. The reversed lactam **58** required the LDA-mediated carboxyethylation of commercially available 4-benzylpyridine with diethylcarbonate toward **56**. Michael addition of acrylonitrile and Raney nickel-mediated reduction of the nitrile produced the lactam **57**. Catalytic reduction of the pyridine to piperidine, followed by coupling of *p*-bromobenzylchloride yielded **58**.

As illustrated in Table 3, replacement of the unsubstituted phenyl in **1** by 2-, 3-, or 4-pyridine (compounds **14–16**) was neutral in terms of receptor potency. The para-position accepts preferably a small substituent such as fluorine in **17** instead of the larger methoxy **19** and carboxylic acid **23**. The meta-position tolerates a variety of substituents of different sizes and polarity such as exemplified by **20**, **22**, and **24–31**. Introduction of solubility enhancing groups as in **24, 27, and 29** at this position is tolerated for activity and could be a good starting point for further optimization of the drug-like properties of this series. The 7-fold potency gain seen for 2,4-fluor analog **18** relative to the 4-fluor analog **17** and the high activity of **21** suggest that an ortho substitution is beneficial for activity.

The contribution of the glutarimide group is shown in Table 4.

The imide hydrogen was substituted by various alkyl groups as in **32–37** without improvement in potency. Retaining one (**54** and **58**) of the carbonyl functions in the glutarimide ring is essential for high activity. The conversion of the glutarimide into a 3-piperidine ring (**55**) results in a decrease of activity, unless the *N*-piperidine atom is, for instance, substituted with a carbonyl-containing residue such as in **41–49**. Sulfonamide **50** is equipotent to the ureum **49**. Highest potency in this series (IC_{50} = 0.06–0.08 μM) was seen

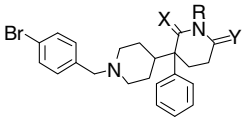
Table 3
hCXCR3 antagonism: modification of the phenyl moiety



Compound	A	R	hCXCR3 binding IC_{50}[a] (μM)
1	C	H	0.78
14	2-Pyridyl	H	0.50
15	3-Pyridyl	H	1.00
16	4-Pyridyl	H	0.63
17	C	4-F	1.26
18	C	2,4-F	0.12
19	C	4-OMe	3.63
20	C	3-OMe	1.55
21	C	2-OMe-5-SO_3H	0.17
22	C	3-Cl	1.45
23	C	4-CO_2H	5.01
24	C	3-CO_2H	0.63
25	C	3-$CONH_2$	3.16
26	C	3-CONHMe	3.16
27	C	3-$CONH(CH_2)_3OH$	1.26
28	C	3-CO-Morpholine	0.79
29	C	3-CO-N-Me-piperazine	1.26
30	C	3-NH_2	0.32
31	C	3-OH	1.00

[a] GTPγS binding assay.[14]

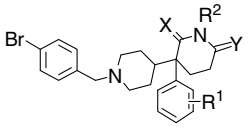
Table 4
hCXCR3 antagonism: modification of the glutarimide moiety



Compound	X	Y	R	hCXCR3 binding IC_{50}[a] (μM)
1	O	O	H	0.78
32	O	O	Me	2.51
33	O	O	Et	2.51
34	O	O	Pr	5.01
35	O	O	*i*-Pr	5.01
36	O	O	$CH_2CH_2OCH_3$	2.00
37	O	O	CH_2SCH_3	1.00
41	H	H	Acetyl	0.14
42	H	H	Ethylcarbamate	0.32
43	H	H	Formyl	0.15
44	H	H	Trifluoroacetyl	0.69
45	H	H	Propionyl	0.29
46	H	H	Cyclopropionyl	0.18
47	H	H	α-Acetamide	0.08
48	H	H	Phenylureum	0.06
49	H	H	Ureum	0.25
50	H	H	Sulfonamide	0.21
54	H	O	H	0.50
55	H	H	H	>10
58	O	H	H	1.58

[a] GTPγS binding assay.[14]

Table 5
hCXCR3 and muscarinic receptor antagonism: enantiomeric effect



Compound	Chirality	X	Y	R^1	R^2	hCXCR3 binding IC_{50}[a] (μM)	Muscarinic receptor binding IC_{50}[b] (μM)		
							M1	M2	M3
1	±	O	O	H	H	0.78			
1a	–	O	O	H	H	0.61	9.6	>10	>10
1b	+	O	O	H	H	1.33	<0.001	<0.001	<0.001
18	±	O	O	2,4-F	H	0.12			
18a	–	O	O	2,4-F	H	0.06	>10	>10	>10
18b	+	O	O	2,4-F	H	0.33	<0.001	<0.001	<0.001
41	±	H	H	H	Acetyl	0.14			
41a	–	H	H	H	Acetyl	0.11	10	10	3
41b	+	H	H	H	Acetyl	2.31	>10	>10	>10
47	±	H	H	H	α-Acetamide	0.08			
47a	–	H	H	H	α-Acetamide	0.81	5	2	1.1
47b	+	H	H	H	α-Acetamide	0.06	>10	>10	>10
48	±	H	H	H	Phenylureum	0.06			
48a	–	H	H	H	Phenylureum	0.03	>10	>10	>10
48b	+	H	H	H	Phenylureum	6.31	8.9	>10	>10

[a] GTPγS binding assay.[14]
[b] Ref. 16 for assay.

with the α-acetamide (**47**) and the phenylureum (**48**) substituents, but at the cost of less favorable PK properties in the case of **48** (data not shown).

Table 5 lists the CXCR3 and muscarinic receptor binding activity of our most potent racemic compounds and their enantiomers. The enantiomers were separated by chiral HPLC or SFC and the enantiomers were distinguished by their optical rotations (levo or dextro rotatory), without further determination of their absolute configuration.

The muscarinic activity of the opposite enantiomer of the hit **1a** lead us to select the enantiomeric pairs of those compounds for screening on a panel of muscarinic receptors in a radioligand binding assay.[16] The isomers of compounds **1** and **18**, which share the same benzetimide (**3**) scaffold, display comparable hCXCR3 antagonistic activities, while their anti-muscarinic activity is almost totally confined to the (+)-isomer form. However, N-substitution of the 3-piperidine ring with carbonyl-containing functions (compounds **41**, **47**, and **48**) indicated that their optical isomers produced weak or no anti-muscarinic activity and that strong CXCR3 antagonistic activity was largely confined to a single enantiomer **41a**, **47b**, and **48a**. Of note, although not tested on a regular basis, our compounds showed comparable potencies against mouse CXCR3 (data not shown), excluding issues of speciation that are often encountered in the search for chemokine receptor antagonists.

Taken together, we have identified a novel series of 3-piperidine compounds that produce submicromolar antagonistic activity against human and murine CXCR3. The compounds were optimized from the HTS hit **1**, a structural analog of the anti-cholinergic benzetimide. From enantiomeric separation of the most potent 3-piperidine compounds it was learnt that CXCR3 antagonism and anti-cholinergic effects were not linked. In vivo experiments are underway to assess the potential of these compounds as anti-inflammatory agents.

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13. Stably transfected hCXCR3-expressing CHO cells were suspended at 10^6 cells/well in DMEM/HAM'S F12 medium supplemented with 10% fetal bovine serum and 1 mM isobutylmethylxanthine. After a 60-min pre-incubation at room temperature with compound, forskolin (30 μM) and ITAC (0.3 μM, Peprotech) were added. After 30 min, intracellular cAMP was measured using the cAMP dynamic kit from CIS bio International.
14. Membranes of hCXCR3-expressing CHO cells were diluted in incubation buffer (20 mM Hepes, 100 mM NaCl, 3 μM guanine diphosphate, 1 mM $MgCl_2$, pH 7.4, 14 μg/ml saponin) and pre-incubated with compound for 30 min at 30 °C in 96-well flashplates (Perkin-Elmer), before stimulation with human ITAC (3 nM) or IP-10 (150 nM) (R&D Systems). [^{35} S]GTPγS (0.25 nM, ~1119 Ci/

mmol, Amersham) was then added and, 30 min later, bound radioactivity was determined on a Topcount (Packard).

15. (a) 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, 2006.; (b) Johnson, M.; Li, A. R.; Liu, J.; Fu, Z.; Zhu, L.; Miao, S.; Wang, X.; Xu, Q.; Huang, A.; Marcus, 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.
16. Muscarinic receptor binding data were obtained from MDS Pharma Services, Bothell, WA 98021, USA.
17. Janssen, P. A. J.; Niemegeers, C. J. E.; Schellekens, K. H. L.; Demoen, P.; Lenaerts, F. M.; Van Nueten, J. M.; Van Wijngaarden, I.; Brugmans *J. Drug Res.* **1971**, *21*, 1365.
18. Human peripheral blood mononuclear cells (PBMCs) were activated with PHA and human IL-2 (R&D Systems) for 3 days and further cultured with IL-2 until the day of analysis (days 9–14). Cells were labeled with Calcein-AM (Invitrogen) and dissolved in HBSS supplemented with 0.2% BSA. After pre-incubation with compound (10 min, 25 °C), 20 μl cell suspension (80,000 cells) was added in 6-fold to the topside of the filter, while the bottom wells of the 96-well chemotaxis chambers (ChemoTx, NeuroProbe) were filled with 3 nM human ITAC. After 3 h at 37 °C, non-migrated cells were removed from the filter and migrated cells were measured using a fluorescent plate reader.
19. PHA/IL 2-activated human PBMC (prepared as above; 2.5×10^5/well) were dissolved in binding buffer (50 mM Hepes, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.02% NaN_3, 0.5% BSA, pH 7.4) and pre-incubated with compound for 30 min, before 80 pM [^{125}I]-ITAC (2000 Ci/mmol, Amersham) was added and kept for 60 min at 4 °C. Cells were harvested upon GF/B filter plates (presoaked in 0.5% polyethyleneimine) and radioactivity was determined by liquid scintillation counting.
20. Chemokine receptor binding data were obtained from MDS Pharma Services, Bothell, WA 98021, USA.
21. a Coesemans, E.; Bongartz, J.-P. A. M.; Van Lommen, G. R. E.; Van Wauwe, J. P. F.; Buntinx, M. WO Patent 090826, 2007.; b Coesemans, E.; Bongartz, J.-P. A. M.; Van Lommen, G. R. E. WO Patent 090836, 2007.