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Towards Small-Molecule CXCR3 Ligands with Clinical Potential

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The CXCR3 chemokine receptor was first discovered in 1996 and has been shown to play an important role in several diseases, most of which are related to inflammation. This review describes in detail the development of small CXCR3 ligands and their therapeutic potential. Classes of CXCR3 antagonists with strikingly variable core structures have emerged. Some of these compounds have confirmed the beneficial role of CXCR3 antagonism in animal models of disease. One of the compounds, AMG487, progressed to Phase II clinical trials but has been withdrawn because of lack of efficacy. New antagonist classes are being developed to reveal the full therapeutic potential of CXCR3.

Introduction

Chemokines are peptides of 70 to 90 amino acids in length which exert important signaling functions in the body. Chemokines are classified into two main subfamilies based on whether the first two of four conserved cysteines are adjacent (CC) or separated by one amino acid (CXC).[1] The chemokine peptides signal via membrane-bound chemokine receptors, whose nomenclature is based on the class of chemokines they bind, that is, CC or CXC chemokine receptors (CCR and CXCR, respectively). The first chemokine receptor was reported in 1991 after the discovery that the chemokine interleukin-8 (now named CXCL8) binds to a class A G protein-coupled receptor (GPCR).[2,3] Chemokine receptors show less than 30% homology with all other known GPCRs and are major modulators in the immune system where they affect, for example, migration of leukocytes. In addition to their role in inflammatory processes, the role of chemokine receptors in organogenesis, angiogenesis, and the central nervous system, but also in metastasis and growth of tumour cells has become apparent.[4,5] Therapeutic interest in chemokine receptors was boosted in 1996 with the finding that chemokine receptors CXCR4 and CCR5 are coreceptors for human immunodeficiency virus (HIV).[6–9] Several chemokine receptors have since attracted interest from the drug discovery community.[10–12] This review will describe one of the promising emerging drug targets of this class, namely the CXCR3 receptor, and will outline all CXCR3-associated medicinal chemistry disclosed to date in scientific and patent literature.

CXCR3 and Peptidergic Ligands

The CXCR3 chemokine receptor was discovered in 1996 during a search for T lymphocyte-specific chemokine receptors. A novel cDNA was isolated from a human CD4+ T cell library and the encoded GPCR proved to have affinity for chemokines.[13] A truncated version of this clone, with an incomplete coding sequence, was already isolated in 1995.[14] CXCR3 consists of 368 amino acids which, as with all GPCRs, are oriented in a typical seven-transmembrane α-helical topology. This is illustrated in detail by the 2D snakeplot in Figure 1. Typical structural motifs for GPCRs such as the conserved DRY motif and the NPxxY$x_{5,6}$F motif at the cytoplasmatic ends of transmembrane domains 3 and 7 respectively are present, as well as cysteine residues in the first and second extracellular loops.[15] Similar to most receptors of the chemokine subfamily of GPCRs,[16] CXCR3 has additional cysteine residues in the amino terminus and third extracellular loop. The threonine and serine residues in the intracellular carboxy tail are potential sites for phosphorylation by receptor kinases.[13,17]

Activated Th1 lymphocytes express high levels of CXCR3, but the receptor is also found on blood T cells and on a small proportion of B cells and natural killer cells.[13,18,19] CXCR3 binds the endogenous CXC chemokines CXCL9, CXCL10, and CXCL11, which before the introduction of the systematic nomenclature[1] were called monokine induced by interferon-γ (Mig), interferon-γ inducible 10-kDa protein (IP-10), and interferon-inducible T cell α chemoattractant/interferon-γ-inducible protein 9 (I-TAC/IP-9), respectively.[13,20–23] Additionally, CXCL13 has been reported to bind and, at high concentrations, activate CXCR3.[24] Chemokine CXCL4 was shown to bind a splice variant of CXCR3 with an extended amino terminus called CXCR3-B,[25] and recently the original CXCR3 (CXCR3-A) has also been reported to mediate CXCL4-induced responses at high concentrations.[26] CXCR3 activates pertussis toxin-sensitive G proteins of the Gα_i class upon activation by chemokines, and mediates chemotaxis, calcium flux, and activation of kinases such as p44/p42 MAPK and Akt.[13,17,27]

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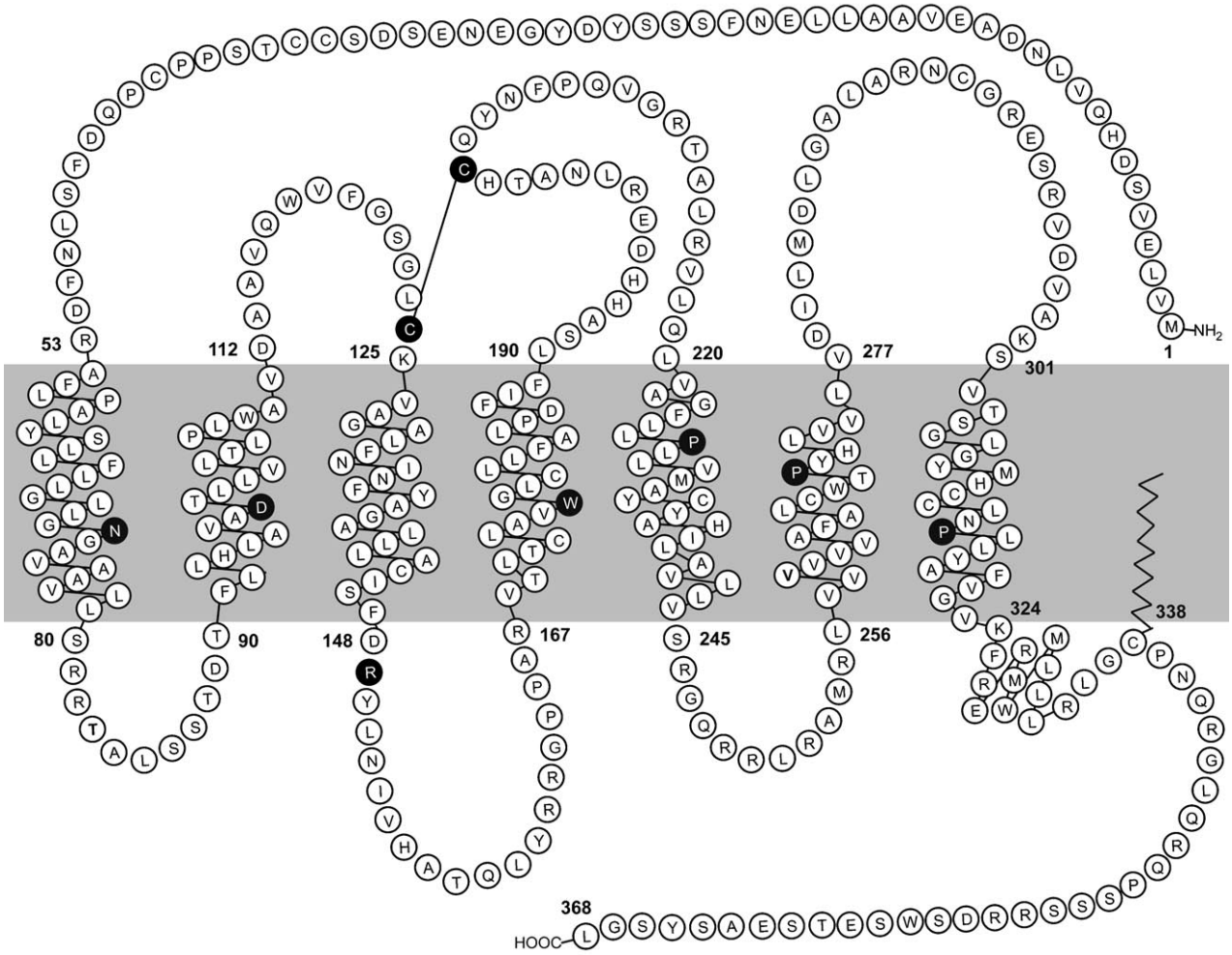


Figure 1. Snakeplot of CXCR3. The typical seven-transmembrane α-helical topology of GPCRs is shown, with an additional proposed helix (helix VIII) in the membrane proximal part of the intracellular carboxy terminus, based on the structure of rhodopsin.[133] Residues that are highly conserved between GPCRs of class A are shown as black residues.[15] These residues are N1.50, D2.50, R3.50 in the DRY motif, W4.50, P5.50, P6.50 in the WxP motif and P7.50 in the NPxxY$x_{5,6}$F motif. In this GPCR numbering according to Ballesteros and Weinstein, the first number refers to the transmembrane helix and the second number indicates the position of the most conserved residue, which is assigned position 50 in that helix.[134] Residues N-terminal from the most conserved residue at position 50 are designated with lower numbers, for example, Asp 148 is assigned D3.49 etc. The use of this Ballesteros-Weinstein numbering simplifies the identification of residues at similar positions between different GPCRs. The presumed disulfide bond between cysteines in the first and second extracellular loops is shown, as well as potential palmitoylation of the cysteine in the carboxy terminus that anchors helix VIII to the membrane.

Although it is common for chemokines to bind several chemokine receptors, this promiscuity is usually restricted to receptors of the same class, that is, CXC chemokines generally bind only to CXC receptors. An intriguing exception may be found for CXCR3: agonists of CCR3, for example, CCL13 and CCL11, bind with high affinity to CXCR3.[21,28] In one study CCL11 blocked CXCR3 activation,[21] although two other studies did not confirm this finding.[28,29] This leaves the question open if CCR3 ligands are true endogenous antagonists of CXCR3 in vivo, or that they may interfere with CXCR3 signalling through other means.

CXCL9, CXCL10, and CXCL11 are all CXCR3 agonists with CXCL11 having the highest potency and efficacy.[13,20–23,30] Typically, the NH_2 terminus of chemokines is important for receptor activation. After binding of a chemokine to the NH_2 terminus and extracellular loops of a chemokine receptor, the NH_2 terminus of the chemokine is believed to interact with yet to be identified domains of the receptor, resulting in activation.[31,32] Consequently, deletion or addition of only a few NH_2-terminal amino acids of the chemokine often changes it from an agonist into an antagonist.[32–34] Indeed, NH_2-terminal truncation of CXCL11 (CXCL11 4-73) barely affects CXCR3 binding affinity, but results in complete loss of agonist activity.[35] Similarly, when the first five amino acids of CXCL10 are deleted and a methionine is added, a potent CXCR3 antagonist is obtained.[36] Interestingly, the endogenous CXCR3 ligands can be processed in vivo by cellular proteases to give chemokines with modified activity. After NH_2-terminal proteolytic processing of CXCL10 and CXCL11 by CD26 (dipeptidyl peptidase IV), the chemokine metabolites lose their CXCR3-mediated chemotactic activity and calcium signalling, while retaining their ability to bind to CXCR3 albeit with reduced affinity.[37,38] CD26-processed CXCL10 inhibits chemotactic responses of CXCR3-expressing cells towards intact CXCL10, illustrating that NH_2-ter-

minal processing of CXCL10 produces a natural CXCR3 antagonist.[38]

CXCR3 as a Potential Drug Target

The general "druggability" of chemokine receptors remains a subject of discussion. Despite the reputation of GPCRs as popular drug targets[39] no antagonists targeting any chemokine receptors have reached the market yet except for HIV entry inhibitors.[11] On the one hand, this may be due to the relatively recent discovery of chemokine receptors. On the other hand, there has been ongoing discussion about the applicability of such antagonists considering the redundancy of the chemokine system. The notion that most chemokine receptors bind more than one chemokine and most chemokines bind to several chemokine receptors clearly complicates the prediction of the therapeutic effects of chemokine receptor antagonists. It is therefore encouraging that specific roles for various chemokine receptors in disease models are emerging.[40] Indeed, based on the upregulated expression of CXCR3 and its ligands, CXCR3 has been implicated in a variety of inflammatory diseases. These include multiple sclerosis,[41] rheumatoid arthritis,[18] atherosclerosis,[42] chronic obstructive pulmonary disease,[43] inflammatory bowel disease,[44] inflammatory skin diseases[22,45] such as psoriasis,[46] hepatitis C infected liver,[47] sarcoidosis,[48] and SARS.[49,50] CXCR3 also appears to be a key factor in the rejection of donor organs after transplantation.[51,52] Moreover, CXCR3 appears to play an important role in metastasis of melanoma and colon cancer cells to the lymph nodes and in metastasis of breast cancer cells to the lung.[53–55] Lastly, for certain HIV virus strains and isolates, CXCR3 may act as a coreceptor.[56]

Various preclinical approaches have been used to confirm the therapeutic potential of the CXCR3 receptor system: 1) the generation of CXCR3 knockout (KO) mice, 2) targeting CXCR3 or its endogenous ligands by antibodies, 3) inhibiting CXCR3 by means of protein-based antagonists, and 4) targeting CXCR3 by small molecules. The first three approaches will be briefly highlighted below, whereas the fourth approach is the topic of the next section.

Use of CXCR3-KO mice

CXCR3-KO ($CXCR3^{-/-}$) mice appear phenotypically normal in the unchallenged host,[51,57,58] although a deficiency in NK cells in the lung and peripheral blood has been reported. Moreover, a reduction of natural killer (NK) and NK T cells in the liver is observed, indicating that CXCR3 is required for NK and NK T cell homeostasis.[59] In murine models of transplant rejection, $CXCR3^{-/-}$ mice showed delayed acute or chronic rejection of cardiac allografts[51] or pancreatic island allografts.[60] In some cases, allografts were even maintained chronically in $CXCR3^{-/-}$ mice, especially in combination with the immunosuppressive drug cyclosporine A.[51] Using $CXCR3^{-/-}$ mice, it was shown that CXCR3 is involved in skin wound healing, although CXCR3 is not a critical factor.[57] Antagonism of CXCR3 signalling is suggested to leave less scarring of the skin, whereas agonism of CXCR3 may result in more rapid maturation of the skin compartments.[57] Studies on $CXCR3^{-/-}$ mice also revealed that CXCR3 plays a critical role in the positioning of effector T cells at sites of viral inflammation in the brain[58] and in limiting lung fibrosis following lung injury.[59]

Targeting of CXCR3 or its endogenous ligands by antibodies

CXCL10 antibodies attenuate chronic experimental colitis by blocking cellular trafficking and protecting intestinal epithelial cells, a finding relevant in diseases such as ulcerative colitis.[61] Notably, a Phase II clinical trial has been launched to investigate a CXCL10 antibody (MDX1100) in treating ulcerative colitis.[62] In addition, similar to the $CXCR3^{-/-}$ models mentioned above, the use of an antibody directed against either CXCR3 or CXCL10 significantly prolongs allograft survival, sometimes even with administration taking place several days after the transplantation.[51,60,63–65] An antibody directed against CXCR3 not only reduced T cell recruitment to inflamed arthritic joints in a rat model of arthritis, but also prevented weight loss by the animals and decreased the severity of arthritis in general.[66] Indeed, the CXCL10 antibody MDX1100 (vide supra) will also be investigated in a Phase II trial for rheumatoid arthritis.[62] Last, a CXCL10 antibody suppressed metastasis of melanoma cells to the lymph nodes in mice.[53]

Although targeting of one or more of the CXCR3 ligands with antibodies appeared beneficial in certain models,[67] targeting CXCR3 appears a more straightforward way to treat the condition as this abrogates the effects of all three chemokines at the same time. Indeed, deletion of either CXCL9 or CXCL10 alone in a mouse model of obliterative bronchiolitis did not affect T-cell recruitment into the allograft, whereas deletion of CXCR3 did.[68]

Targeting of CXCR3 by protein antagonists

The strategic use of protein-based antagonists (for example, chemokine analogues) has confirmed some of the key roles presented so far. In a mouse model for skin inflammation, CXCL11-based antagonists reduced swelling of the skin in response to a sensitiser.[69] Such antagonists also inhibited neuroinflammation in mice implanted with the neurotoxic CXCR3-binder SDF(5-67).[70] Administration of CXCL10-based protein antagonists to mice reduced the progression of autoimmune sialadenitis, which relates to the inflammation of the salivary glands as observed in Sjögren's syndrome.[36]

Targeting of CXCR3 by Nonpeptidergic CXCR3 Antagonists

Figure 2 shows that publications and patents on small CXCR3 ligands first emerged around 2001 with an increase occurring in almost all subsequent years. As there is only a handful of speculative therapeutic indications for CXCR3 agonists (vide supra), virtually all the reports deal with antagonists. The CXCR3 antagonist area has been reviewed before.[71,72] The current review provides the latest developments in this area, in-

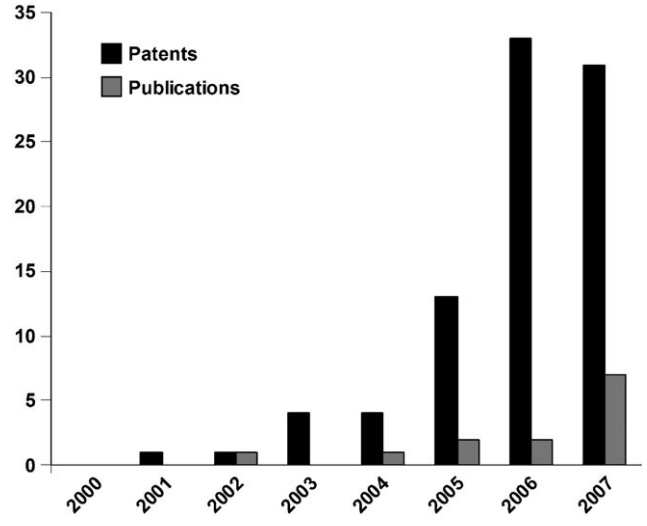
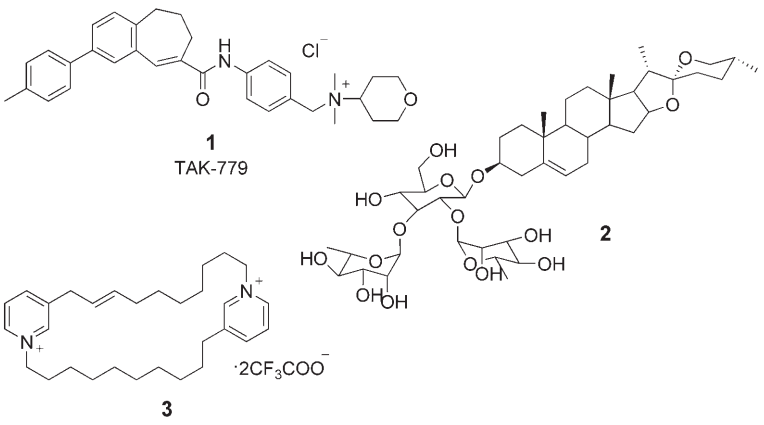


Figure 2. Numbers of patents and publications that have appeared on structure–activity studies of small CXCR3 ligands per year. Patents: Numbers were obtained by searches with SciFinder Scholar and EspaceNet. International Filing Date was used. Publications: Numbers were obtained by detailed searches with SciFinder Scholar and PubMed and do not include conference reports. Date of acceptance was used.

cluding the spark in publications that has occurred in recent months. Below, all known classes of CXCR3 antagonists will be discussed with accompanying medicinal chemistry and available (pre)clinical data. In all cases, affinity or activity numbers are accompanied by the reference chemokine and/or the type of assay used (if reported) and, unless specified, human CXCR3 was used in these assays.

TAK-779 and naturally occurring nonpeptidergic antagonists

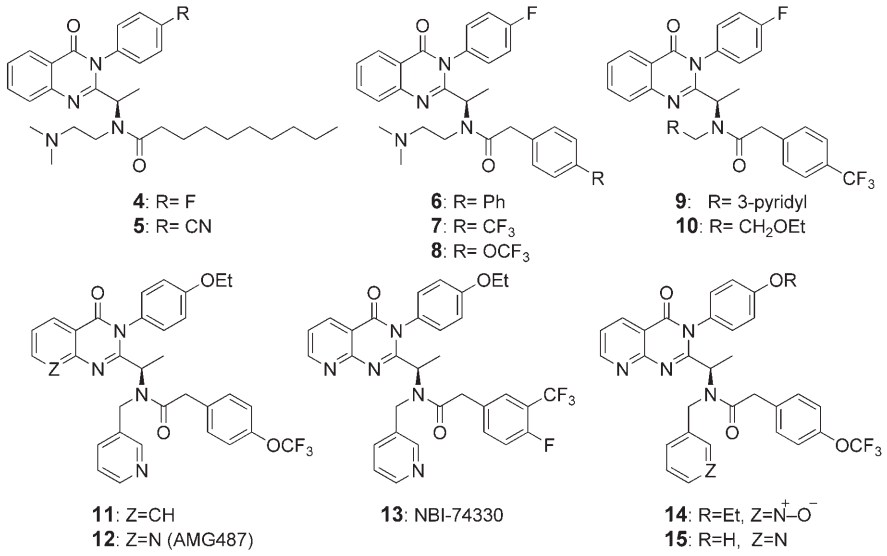
In addition to the frequently used method of screening corporate collections, two other routes have afforded CXCR3 hits. First, the known CCR5-ligand TAK-779 (**1**)[10] attracted some interest from the CXCR3 community as it proved to also bind mouse CXCR3 (IC_{50} = 369 nM, ^{125}I-CXCL10).[73] However, despite showing efficacy in rodent models involving CXCR3, CCR5, and CCR2,[74–77] the moderate affinity of TAK-779 for CXCR3 and its poor selectivity profile have rendered it only of limited value to CXCR3 research as a whole. Secondly, various natural products were found to bind CXCR3. Merck performed a screen (^{125}I-CXCL10) on a library consisting of extracts from microbial, plant, and marine sources.[78] A highly diverse set of hits was picked up, including sugar-derivatised steroid **2** (IC_{50} = 470 nM) and dipyridinium salt **3** (IC_{50} = 690 nM).



(Aza)quinazolinones—from bench to clinical trials

The companies Chemocentryx and Tularik, later acquired by Amgen, teamed up to develop small (aza)quinazolinone-based CXCR3 antagonists leading to an array of patents with the first one appearing in 2001.[79,80] Compound **4** was retrieved as a moderate hit (IC_{50} = 250 nM, ^{125}I-CXCL10) from a high throughput screening (HTS) campaign, but it displayed unacceptable pharmacokinetic properties.[81] Studies on **4** identified the decanoyl and dimethylamino group as major metabolic culprits.[81] However, replacement of the decanoyl chain in **4** by other hydrophobic groups initially led to compromised affinity.[81,82] Later, it was discovered that a biphenylmethylene group (**6**) or an isostere with a *p*-CF_3 (**7**) or *p*-OCF_3 group (**8**) could be successfully introduced.[81] With improved hit **7** in hand, the dimethylamino group was investigated. Other groups, such as a 3-pyridylmethyl (**9**) and 2-ethoxyethyl (**10**), served as effective substitutes.[81] In many compounds, the 4-F group can be successfully exchanged for a 4-CN group.[80] For example, applying such an exchange on compound **9** gave maintained affinity,[81] whereas for **4** it was found to give a threefold boost in affinity (that is, **5**, VUF5834).[82] The affinity of **9** could be improved further by substituting the 4-F atom by a propargyl or ethoxy group.[80,81] Eventually, the pharmacokinetically more attractive 4-ethoxy substituent was combined with the CF_3O-substituted phenylacetamide moiety to deliver **11** (IC_{50} = 6 nM, ^{125}I-CXCL10). Seeking an increase in polarity of compound **11**, an N atom was introduced in the Ph ring of the quinazolinone to give azaquinazolinone **12** (IC_{50} = 8 nM, ^{125}I-CXCL10).[80,81] Compound **12**, dubbed AMG487, contains one chiral centre which has the (*R*)-configuration. This configuration is important for affinity, as the (*S*)-enantiomer is less efficient.[83] AMG487 is currently the most studied member of the azaquinazolinone class. In addition, a more active 4-F, 3-CF_3 analogue (**13**, NBI-74330) from the same patent[80] was independently studied by researchers from Neurocrine and UCB (K_i = 1.5 nM, ^{125}I-CXCL10).[84,85]

The (pre)clinical properties of NBI-74330 and AMG487 have been extensively studied. NBI-74330 inhibits CXCL11 in [^{35}S]-GTPγS binding (IC_{50} = 10.8 nM), Ca^{2+} mobilisation (IC_{50} = 7 nM), and chemotaxis (IC_{50} = 3.9 nM).[84] The antagonism of human CXCR3 is noncompetitive, that is, the maximum signal induced by CXCL11 (E_{max}) was dose-dependently reduced by NBI-74330 with notable reductions already visible at 3 nM ([^{35}S]-GTPγS). Our group has confirmed noncompetitive antagonism on human CXCR3 by NBI-74330, amongst other antagonists.[86] Noncompetitive antagonism for NBI-74330 is also reported on murine CXCR3 (pA_2 = 8.35, CXCL11, [^{35}S]-



GTPγS), but in a less pronounced manner.[85] Here, NBI-74330 induced a right shift in the EC_{50} at low and high concentrations, but only a significant reduction in E_{max} at high concentrations (1 μM).

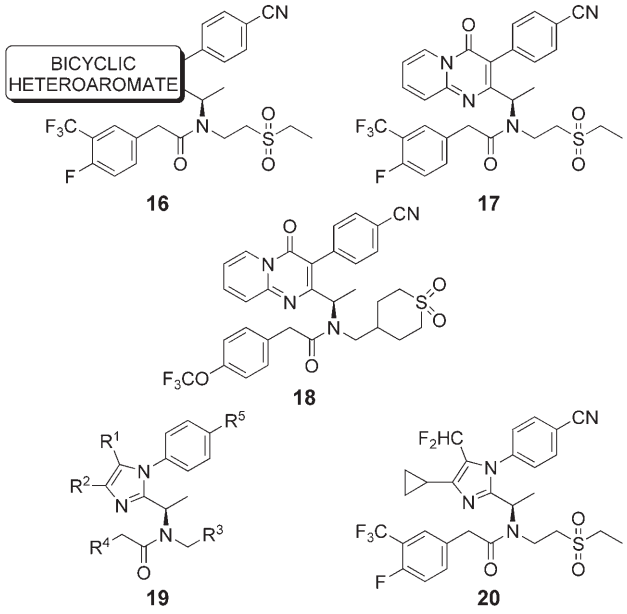
Similar to NBI-74330, the structurally related AMG487 exhibits noncompetitive antagonism.[86] It inhibits CXCR3-mediated cell migration (IC_{50} = 15 nM, CXCL11) as well as Ca^{2+} mobilisation (IC_{50} = 5 nM, CXCL11).[81] Interestingly, in addition to this antagonism of the original CXCR3 (CXCR3-A), AMG487 also inhibits CXCL4 and CXCL11-mediated responses through the alternatively spliced variant CXCR3-B.[26] The compound displays a greater than 1000-fold selectivity for CXCR3 versus a panel of other receptors, including 11 chemokine receptors.[87] Compared to initial HTS hit **4**, AMG487 has lower clearance (1.6 and 1.1 $L\,h^{-1}\,kg^{-1}$, 0.5–1.0 $mg\,kg^{-1}$ i.v. in rats and dogs, respectively) and an improved bioavailability (12-57 and 85%, 2.0–2.5 $mg\,kg^{-1}$ orally in rats and dogs, respectively).[81] The safety profile of AMG487, as assessed by various genotoxicity and cardiotoxicity assays, revealed no major concerns.[83] The two main metabolic pathways for AMG487 involve CYP3A4-mediated oxidation of the pyridine N-atom to the N-oxide (**14**) and de-ethylation to phenol **15**. Metabolite **14** efficiently binds CXCR3 (IC_{50} = 6 nM, ^{125}I-CXCL10)[83] and has also been patented.[88] The area-under-the-curve (AUC) ratio of **14** versus AMG487 varies from 0.03 to 0.6 in various animal studies.[83] Recent studies on analogue NBI-74330 have shown that this ratio also depends on the mode of administration. Higher exposure of NBI-74330 over N-oxide was achieved by oral dosing, whereas subcutaneous (s.c.) dosing led to about equivalent exposures.[85] It may be expected that similar dependences on administration hold true for AMG487.

Studies with NBI-74330 and AMG487 in animal models reveal that this azaquinazolinone class of CXCR3 antagonists bears clinical promise in a variety of diseases. The ability of AMG487 to inhibit inflammatory cell migration in vivo was confirmed in a mouse model of bleomycin-induced cellular recruitment, where AMG487 significantly reduced infiltration of macrophages and lymphocytes into the lungs with infiltration levels being as low as in CXCR3-KO mice (3 $mg\,kg^{-1}$ sc).[83] In a mouse model for idiopathic pneumonia syndrome (IPS), AMG487 reduced recruitment of donor T cells to the lung after allogeneic stem cell transplantation, leading to improved survival rates.[89] Likewise, reductions in inflammation, pannus formation, and cartilage damage were observed upon administering AMG487 at doses up to 50 $mg\,kg^{-1}$ s.c. in mouse collagen-induced arthritis models.[90] Interestingly, NBI-74330 gave rise to a reduction in lesion formation in models for atherosclerosis by inhibition of effector cell migration to the atherosclerotic plaque and by regulating the local immune response.[91] Last, as outlined earlier, metastasis of breast cancer was identified as a possible therapeutic area. This was substantiated by the inhibiting effect of AMG487 on lung metastasis in a murine model for metastatic breast cancer.[54]

The preclinical studies convincingly paved the way for clinical studies on two inflammation-related diseases: psoriasis and rheumatoid arthritis. In 2003, results of a Phase I trial on AMG487 were disclosed. The compound was assessed for safety and pharmacokinetics in 30 healthy males in a randomised, double blind, placebo-controlled dose-escalation study. Generally, the compound was well tolerated and adverse events were mild to moderate (25 to 1100 mg doses).[92] In a subsequent Phase IIa trial, patients suffering from psoriasis received 50 or 200 mg of AMG487 or placebo orally once a day for 28 days. Disappointingly, no significant differences in the endpoints (psoriasis severity index or physician global assessment scores) were seen between patient groups. It was speculated that this lack of clinical efficacy may result from high variability in drug exposure.[93] Metabolic studies with healthy humans provided a plausible explanation for such variability.[94] Of key relevance were the two metabolites **15** and **14**, the latter being formed through CYP3A4. The studies revealed that **15** was a relatively potent (5 μM) and time-dependent inhibitor of CYP3A4, leading in turn to variable formation of the major metabolite **14**. In 2004, it was announced that Phase II trials with AMG487 on patients with rheumatoid arthritis were to be initiated.[95] The current status of these trials is unknown.

It comes therefore as no surprise that the latest lead optimisation efforts on AMG487 have aimed at replacing the metabolically liable pyridine ring, ethoxy group, and azaquinazolinone core.[96–98] The described N-oxidation can be blocked through replacement of the pyridine ring by a sulfone group. Likewise, metabolic de-ethylation can be circumvented by replacing the ethoxy group with a cyano group.[97,98] Changing the azaquinazolinone bicyclic core for a wide variety of hetero-

cyclic groups (**16**) was tolerated and sometimes beneficial, leading to the hypothesis that the rigid bicyclic core serves as a scaffold to hold the adjacent groups in the correct orientations.[98] These structural replacements differentially affected



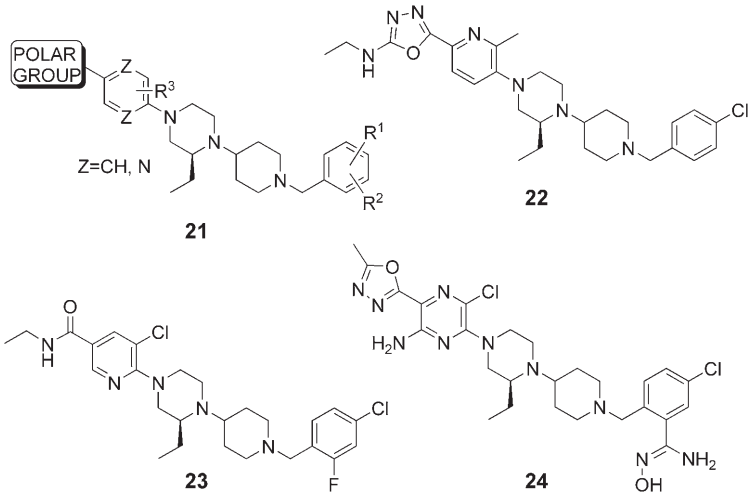
binding to plasma proteins and the overall effects had to be balanced. This led to the identification of **17** which compared to AMG487 had similar affinity but reduced clearance (0.24 $L\,h^{-1}\,kg^{-1}$, 0.5 $mg\,kg^{-1}$ i.v. in rat).[98] However, compounds in this class of pyrido-[1,2-a]pyrimidin-4-ones appear to suffer from CYP-induction mediated by the pregnane X receptor. It was reported that optimisation to structure **18** could counteract this unwanted effect.[96] Another effective replacement of the azaquinazolinone core is an imidazole group (**19**) substituted at the 4-position (R^2) with a lipophilic group.[97] Although affinities could be kept in the low nM range within this class, it presented its own metabolic hurdle: substantial addition of glutathione to the imidazole ring. This was elegantly overcome by installing electron-withdrawing groups on the 5-position of the imidazole ring (R^1 in **19**) as exemplified by compound **20** ($IC_{50}=18$ nM, ^{125}I-CXCL10), although clearance levels remained inferior to those of **17** (**20**: 2.2 $L\,h^{-1}\,kg^{-1}$, 0.5 $mg\,kg^{-1}$ i.v. in rat).

Piperazinylpiperidines

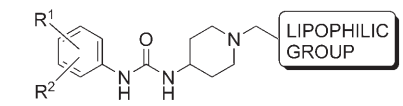
Schering–Plough has filed many patents describing a piperazinylpiperidine-scaffold flanked by a substituted benzyl unit and a polar head group (general structure **21**). Notably, many compounds of this class have subnanomolar affinities (for example, **22**, **23**, **24**: $K_i=0.2$ nM, ^{125}I-CXCL10).[99–101] Upon inspection of the best compounds, the (*S*)-configuration of the ethyl-substituted carbon is maintained and R^1 and/or R^2 in **21** are often halogens or halogenated groups, suggesting crucial roles for these moieties. No functional data has been disclosed.

1-Aryl-3-piperidin-4-ylureas

UCB has designed CXCR3 antagonists based on an initial rigid piperidinylurea scaffold (general structure **25**). A HTS campaign using a FLIPR-based calcium flux assay led to the identification of hit **26** ($K_i=110$ nM, [^{35}S]-GTPγS). This compound was reported to have poor solubility (0.1 $\mu g\,mL^{-1}$).[102] Replacement of the cyclooctenyl ring by a variety of highly lipophilic substituents mostly afforded K_i values higher than 10 μM. A fortunate exception was the naturally occurring (−)myrtenyl group which gave a compound with affinity similar to **26**.[102] With the (−)myrtenyl group in place, extensive structural variation of the aromatic group was performed to optimise affinity and druglike properties. Several compounds were identified that had better affinities ([^{35}S]-GTPγS) and improved physicochemical properties compared to hit **26**. This can be clearly illustrated by compound **27** ($K_i=16$ nM, solubility 23 $\mu g\,mL^{-1}$). N-methylation of **27** to a quaternary ammonium salt was allowed for the CXCR3 interaction while it further increased solubility. However, the resulting compounds suffered from reduced membrane permeability.[102,103] To improve the in vivo pharmacokinetic properties of **27**, two approaches were followed. In the first approach, the urea group was replaced by a hydan-



toin or imidazolinone group and by a modelling-inspired switch to a benzazole or aryl azole.[104] None of these alternative linkers surpassed **27** in affinity, but several gave increased microsomal stability and low CYP inhibition. For example, **28** displayed low clearance (2.8 $mL\,min^{-1}\,kg^{-1}$) and a long plasma half-life (5.4 h). A second and seemingly more successful approach focused on the spacer and (−)myrtenyl group in **27**.[105] One hundred compounds with myrtenyl replacements were prepared, affording terminal piperidinylamides (**29**) as pharmacokinetically more favourable compounds. Subsequent modelling studies suggested that a homotropenylamide would spatially better resemble the myrtenyl group in **27** than an unsub-



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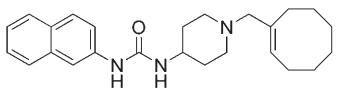

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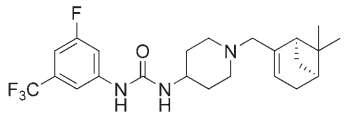

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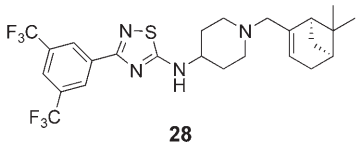

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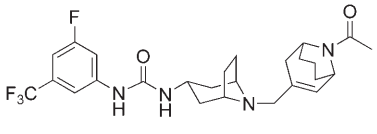

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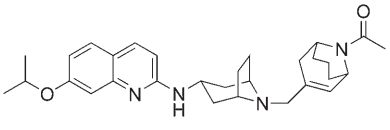
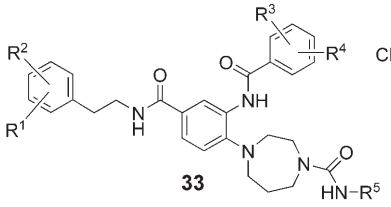


32

stituted piperidinylamide in **29**. Concomitant focus was placed on preventing the central aminopiperidine unit from being oxidised in vivo. Interestingly, modelling revealed that, once again, a tropanyl unit would do just that by bridging the ring. Thus, an *exo*-tropanyl central core and homotropenyl peripheral unit were combined to deliver **30**. Compared to **27**, this compound is slightly more potent ($K_i = 7$ nM, [^{35}S]-GTPγS) but is cleared less rapidly (7 μL min^{-1} mg^{-1}), boosts a similar solubility (40 μg mL^{-1}), and has a high bioavailability (70%). Notably, a high selectivity for CXCR3 in a panel of 50 receptors was disclosed for **30**.[105] With the novel (homo)tropanyl-type structural elements at hand, replacement of the urea group was revisited.[106] This resulted in the discovery of quinoline-based antagonists (**31**). Compared to **30**, one isopropoxy-substituted member (**32**) has comparable affinity ($K_i = 5$ nM, [^{35}S]-GTPγS) and higher solubility (1280 μg mL^{-1} at pH 6.5), but at the same time an increased propensity to bind plasma proteins. Quinoline **32** was tested for its in vivo properties where it showed good oral availability ($t_{1/2} = 7.6$ h, 30 mg kg^{-1} p. o. in mice) and dose-related inhibition of CXCR3 internalisation.[85] At 100 mg kg^{-1}, an effect on CXCR3 internalisation was observed up to 24 h post administration.

4-*N*-aryl-[1,4]diazepanylureas

Pharmacopeia researchers screened over four million compounds (90 libraries)[107] using a FLIPR-based calcium mobilisation assay.[108,109] This HTS screen led to the discovery of various antagonist scaffolds.[108] The general structure of one disclosed class is represented by **33**. The phenethyl substituent benefits from halogen substitutions, most particularly a 3,5-dichloro pattern. A similar preference for a 3-chloro (but also 3-fluoro) substituent was observed for the benzamide moiety. Replacement of the azepane spacer or the urea unit by different groups led to a dramatic drop in affinity. The combined SAR studies resulted in the discovery of **34** as a potent CXCR3 antagonist ($IC_{50} = 60$ nM, CXCL11, Ca^{2+}).[108] The compound was capable of inhibiting chemotaxis ($IC_{50} \sim 100$ nM, CXCL11). No cytotoxicity at 100 μM was observed whereas high selectivity over 14 other GPCRs was noted.



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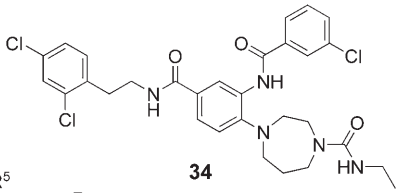

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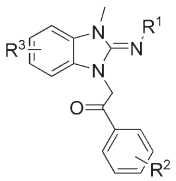

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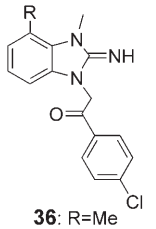


36: R=Me
37: R=Et

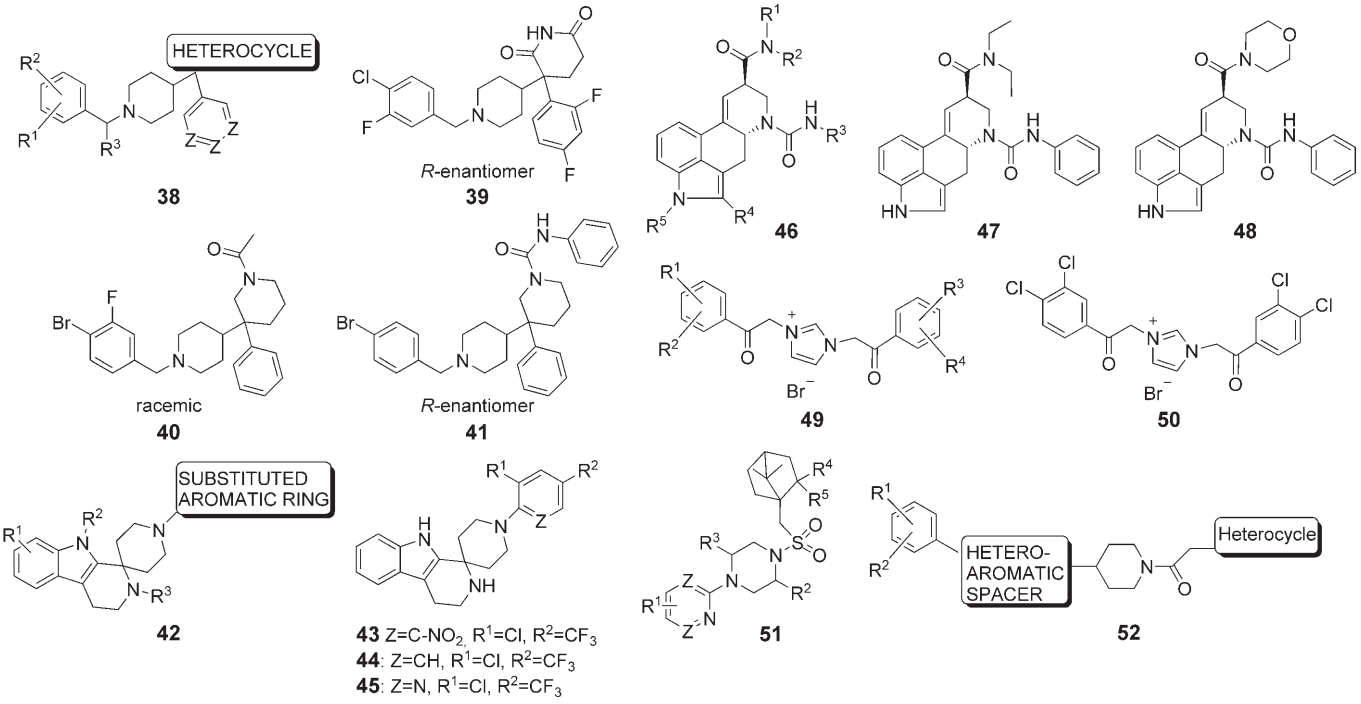
2-Iminobenzimidazoles

Researchers from Abbott Laboratories recently disclosed 2-iminobenzimidazoles as CXCR3 antagonists.[110,111] The initial HTS hit, a substituted 2-acetyl-1*H*-benzo[*d*]imidazole, and several first-generation derivatives gave reasonable affinities ($IC_{50} = 2$–20 μM, ^{125}I-CXCL10) but suffered from solubility problems in aqueous buffer. This complicated the pharmacology as it gave apparent partial antagonism. A strategic substitution of the 2-acetylbenzimidazole core by a 3-methyl-2-iminobenzimidazole moiety (general structure **35**) afforded not only much improved solubility but also higher affinities. Variation of the benzophenone substituent (R^2) in this scaffold showed that 4-halo substituents gave submicromolar affinities. Upon selection of the 4-Cl group, the effect of ring substituents at the benzimidazole core (R^3) was investigated. The most notable improvements in affinity resulted from installing small, apolar substituents at the C4 position (**36**: $IC_{50} = 100$ nM, **37**: $IC_{50} = 30$ nM/^{125}I-CXCL10). Whereas this represented an 8- and 27-fold boost in affinity for **36** and **37** compared to the unsubstituted counterpart (R = H), the corresponding boost in functional antagonism was an interesting 113- and 129-fold, respectively (**36**: $IC_{50} = 80$ nM, **37**: $IC_{50} = 70$ nM/CXCL10, Ca^{2+}). Compound **36** was

evaluated for its in vivo pharmacokinetic properties, where it showed a $t_{1/2}$ of 4.9 h and a bioavailability of 57% upon oral dosing in mice (10 mg kg^{-1}).

Bispiperidines

Researchers from Janssen have devised compounds centred around the 3,4′-bispiperidine scaffold (**38**).[112,113] One of the N-termini was linked to an amide or urea group (3,4′-bispiperidinylamides and -ureas), or carbonyl groups were included in one of the piperidine rings (3,4′-bispiperidine-2,6-diones). The



scaffolds were decorated by Ph rings, with ring halogenation often appearing to be a privileged manipulation. Exemplary compounds from all three series are represented by structures (*R*)-**39**, **40**, and (*R*)-**41** (IC_{50} = 79, 50, and 32 nM, respectively, CXCL11, [^{35}S]-GTPγS). Within this class of compounds, the (*R*)-configuration seems generally preferred, which can be deduced from selected affinities of (*S*)-isomers reported in the same patents ((*S*)-**39:** IC_{50} = 251 nM, (*S*)-**41** IC_{50} = 6310 nM).[112,113] Two piperidine rings were also the essence of a patent filed by Amgen, but there the connection was established through a spiro-fusion (**42**). This novel scaffold was discovered after a screening of Amgen's chemical library.[114] The compounds were decorated with a fused indole and halogenated aromatic rings. Compounds **43**–**45** all had IC_{50} values < 500 nM as reported in the patent.[115]

Ergolines

A rather unusual type of CXCR3 antagonist was patented by Roche (**46**).[116] Replacement of the N-Me group of LSD by an N-phenylcarbamate moiety afforded compound **47**. It exerts good binding (IC_{50} = 54 nM, ^{125}I-CXCL11) and blocks Ca^{2+} mobilisation (IC_{50} = 18 nM, CXCL11) and chemotaxis (IC_{50} = 74 nM, CXCL11). Slight improvements could be achieved by certain replacements of the diethylamide moiety. For example, morpholine-containing compound **48** has an IC_{50} of 23 nM (^{125}I-CXCL11) but otherwise similar functional properties as **47**. This class of compounds successfully reduced vessel wall remodelling after allotransplantation in murine models.[116] This is to our knowledge the first SAR report on ergoline-type compounds for a chemokine receptor, although these are known as promiscuous GPCR ligands.[117]

Various

A handful of additional different scaffolds for CXCR antagonists have been disclosed in patents but often with little pharmacological data. As a result, no clear SAR can be deduced. To offer the reader the fullest overview possible, we show herein the general structure of these scaffolds as deduced from inspecting of all structures.

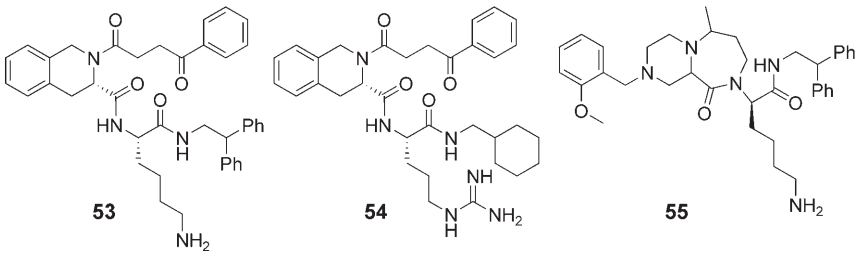
Charged imidazolium salts (**49**) were reported by SmithKline-Beecham researchers.[118] One such compound (**50**) has a K_i value of 251 nM (^{125}I-CXCL10).[86] This group also disclosed camphor-containing antagonists of structure **51**, which had potencies up to 10 nM (CXCL10, Ca^{2+}).[119] Three patents from Merck describe a substituted piperidinylamide linked by its C4 position to a substituted Ph ring through a heteroaromatic spacer such as a thiazole or pyridine (**52**).[120–122] Reported IC_{50} values were as low as 0.5 nM (CXCL10, chemotaxis).

Nonpeptidergic CXCR3 Agonists

Intuitively, development of agonists for inflammatory chemokine receptors does not seem attractive from a therapeutic point of view.[123] However, CXCR3 may offer an intriguing ex-

ception. One study suggests that CXCR3 agonism is beneficial in skin wound healing (vide supra).[57] Moreover, the CXCR3 agonists CXCL9,[124] CXCL10,[125] and CXCL11[126] have been shown to possess antitumour activity, which is attributed to the recruitment of leukocytes by these chemokines. Therefore, topical application of a CXCR3 agonist may have beneficial effects in these specific cases.

In itself the task of designing a small nonpeptidergic activator for any chemokine receptor seems daunting. Nevertheless, a handful of agonists for chemokine receptors other than CXCR3 has already been found in recent years.[127–129] CXCR3 agonists were disclosed by researchers from Pharmacopeia in 2006. In an HTS screen for antagonists of a pool of more than four million compounds, they identified a few CXCR3 agonist chemotypes.[109] Three exemplary compounds (**53**–**55**) were described in detail.[109] All three show structural similarities: a basic amino acid, a hydrophobic group, and an N-containing bicyclic unit. Notable differences include the lack of a benzopropione unit in **55** and the opposite stereochemistry of the amino acid in **55** compared to **53** and **54**. Compounds **53**–**55** activate CXCR3 ($EC_{50}=3.3$, 1.1, and 1.7 μM, respectively / Ca^{2+} influx) with high efficacies (120, 120, and 100% of that of CXCL11, respectively). Activation by the agonists was dose-dependently inhibited by antagonist **34**, indicating specific CXCR3-mediated effects. This was further illustrated by lack of binding to a panel of GPCRs, including six chemokine receptors. Importantly, **54** and **55** were able to stimulate chemotaxis of T-cells in vitro (no results for **53** disclosed).



53 **54** **55**

Modulation of CXCR3 by Nonpeptidergic Ligands. General Considerations

Structural elements

The diversity amongst the CXCR3 antagonists described in this review is quite high and a general pharmacophore model seems difficult to construct. Indeed, to date no such models have been proposed in the public domain. Basic or charged groups are often thought to be beneficial for chemokine receptor affinity. Whereas a good deal of the discussed antagonists possess permanent charges or basic groups poised for protonation at pH 7.4, more and more emerging ligands lack a highly basic group (see **18**, **34**, and **47**). Also of importance is that noncompetitive CXCR3 antagonism occurs in a structurally diverse set of compounds.[84–86] Given the huge structural and spatial differences between the small antagonists and large chemokines, their binding sites are likely different. Precedence for such differential binding of chemokine ligands can be found; for example, TAK-779 was shown to bind CCR5 in a cavity between transmembrane helices I, II, III, and VII rather than at the extracellular domain.[130] Lastly, CXCR3 antagonists of different structural classes have been shown to act as inverse agonists at a constitutively active mutant of CXCR3, namely CXCR3 N3.35A.[86]

A recurring and highly important issue in drug research is the translation of animal models to human studies. Chemokine research represents no exception to this. For example, for the CXCR2 receptor, mouse knock-in models have been specifically constructed to circumvent problems related to species differences.[131] Another illustration involves TAK-779, which has a 100-fold higher affinity for human CCR5 than for mouse CCR5, complicating interpretation of the results from murine studies.[73,132] A careful inspection of all available results on CXCR3 points towards some species differences, caused by differences in the protein sequence of CXCR3 from various species. Compound **27** has affinities of 16 and 227 nM for human and murine CXCR3 ($[^{35}S]$-GTPγS),[102] respectively, whereas some compounds of the related later-stage tropanyl class reveal a 3–4-fold preference for human CXCR3.[105] A systematic study on other antagonist classes (AMG487, NBI-74330, **5**, and **50**) shows a similar fourfold higher affinity for human and rhesus macaque CXCR3 compared to rat or mouse CXCR3.[86] Clearly, there is a slight CXCR3 species difference but it is believed that it does not represent a serious hurdle for future CXCR3 drug discovery efforts.

(Pre)clinical effects

Inhibiting the recruitment of inflammatory cells is at the heart of the clinical rationale for developing CXCR3 antagonists. Animal models using AMG487 and NBI-74330 suggest that this rationale bears fruit. That is, CXCR3-related therapeutic effects have been observed in a general model for in vivo recruitment of inflammatory cells[83] and in more specific models for idiopathic pneumonia syndrome,[89] arthritis,[90] and atherosclerosis.[91] Unfortunately, no beneficial effect was observed with AMG487 in Phase IIa trials on psoriasis. This clinical failure may have been due to pharmacokinetic properties rather than pharmacodynamic properties. Clinical promise of CXCR3 antagonism therefore remains to be confirmed by newer generations of compounds.

Conclusion and Outlook

This review has dealt with the different aspects of CXCR3 as a drug target with emphasis on the potential of small, nonpeptidergic ligands to therapeutically modulate the receptor. After a relatively slow start, more and more ligand classes are steadily disclosed by the drug discovery community. The structural variability amongst these classes is strikingly high. One quest for

antagonists has left researchers with the first series of small molecule CXCR3 agonists, which will undoubtedly prove useful as research tools. For the antagonists, the highest affinities are found for the piperazinylpiperidines from Schering–Plough. In contrast, the best described series are the 1-aryl-3-piperidin-4-ylureas from UCB and most notably the Amgen class represented by AMG487 and NBI-74330. Positive preclinical results with the latter two CXCR3 antagonists have strengthened the therapeutic expectations for CXCR3 antagonism. Unfortunately, a Phase IIa clinical trial with AMG487 has been halted. As this may have been due to unacceptable variability in drug exposure, it is clear that this failure is not a falsification of CXCR3 as a drug target per se. Indeed, clinical promise for the CXCR3 system is illustrated by the recent announcement of two Phase II clinical trials investigating a CXCL10 antibody in treating ulcerative colitis and rheumatoid arthritis.[62] In all, CXCR3 target validation in humans still remains the ultimate and elusive goal and it is expected that ongoing medicinal chemistry efforts will soon shed more light on the therapeutic use of small CXCR3 ligands.

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Keywords: chemokines · CXCR3 · ligands · medicinal chemistry · receptors

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