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# Designing small molecule CXCR3 Antagonists

## Abstract

### Introduction

By virtue of its specificity for chemokines induced in Th1-associated pathologies, CXCR3 has attracted considerable attention as a target for therapeutic intervention. Several pharmacologically distinct small molecules with *in vitro* and *in vivo* potency have been described in the literature, although to date, none have shown efficacy in clinical trials.

### Areas covered

In this article I will outline the rationale for targeting CXCR3 and discuss the potential pitfalls in targeting receptors in poorly understood areas of chemokine biology. I will also cover emerging therapeutic areas outside of the "traditional" Th1 arena in which CXCR3 antagonists may ultimately bear fruit. The design of recently discovered small molecules targeting CXCR3 will be also be discussed.

### Expert Opinion

CXCR3 and its ligands appear to play roles in a multitude of diverse diseases in humans. *In vitro* studies suggest that CXCR3 is inherently "druggable" and that potent, efficacious small molecules targeting CXCR3 antagonists will find a clinical niche. However, the well-trodden path to failure of small molecule chemokine

receptor antagonists in clinical trials suggests that a cautious approach should be undertaken. Ideally, unequivocal evidence elucidating the precise role of CXCR3 should be obtained before targeting the receptor in a particular disease cohort.

**Keywords**

Chemokine receptor, Chemokine receptor antagonist, CXCR3, inflammation.

## 1. Introduction

Chemotactic cytokines (chemokines) are a family of around 40 small proteins in the human which bind to specific G protein-coupled receptors (GPCRs) expressed by a variety of cells, notably leukocytes. Upon receptor binding, several intracellular pathways are induced in the leukocyte which typically results in actin remodeling and migration of the cell towards the source of the chemokine (chemotaxis). Inadvertent or over expression of chemokines has been noted in the inflammatory components of many clinical important diseases, including asthma, atherosclerosis and arthritis. Since GPCRs are deemed “highly druggable” by small synthetic molecules, blockade of leukocyte migration by interfering with chemokine receptor signaling is an attractive therapeutic angle which has been pursued by both academia and the pharmaceutical industry.

The chemokine receptor CXCR3 is expressed by several different leukocytes, notably activated T lymphocytes, NK cells and endothelial cells [1-3]. It is the principal signaling receptor for the CXC chemokines CXCL9, CXCL10 and CXCL11 which are associated with Th1-mediated adaptive responses against intracellular pathogens such as bacteria and viruses. The CXCR3 axis has been suggested as

playing an important role in several human diseases, with expression of CXCR3 and its ligands implicated in rheumatoid arthritis (RA) [3], multiple sclerosis [4], transplant rejection [5] and atherosclerosis [6]. Although around 40% of freshly isolated T cells are $CXCR3^+$ positive, they are poorly responsive to CXCR3 ligands. However, upon exposure to IL-2 and mitogenic stimuli *in vitro,* the percentage of expressing cells rises to almost 100% with all three ligands inducing significant chemotactic responses from the cells [2, 7]. Activation of the receptor appears to have a complex network of regulation, with an early study from scientists at Schering-Plough describing the ligands as allotropic, with G protein coupling influencing which ligands CXCR3 would bind [8]. CXCR3 signaling was subsequently examined in mice deficient in the $G\alpha_{i2}$ and $G\alpha_{i3}$ G protein subunits, revealing an absolute requirement for $G\alpha_{i2}$ and an inhibitory role for $G\alpha_{i3}$ [9]. Also peculiar for a chemokine receptor is the obligatory degradatory fate of endocytosed CXCR3 with an absence of receptor recycling [7]. This is thought to negatively regulate the potential for forward feedback since Th1 cells are a source of IFNγ which is noted for its induction of CXCR3 ligands.

## 2. CXCR3 and its blockade in disease – Success and failure

A number of small molecule CXCR3 antagonists were identified and reported in the literature between 2003 and 2009 and have been previously described in the sister journal “Expert Opinion on Therapeutic Patents” [10]. Many of these compounds possessed nanomolar potency *in vitro* but suffered from poor *in vivo* pharmacokinetics. For those molecules with reasonable bioavailability, the fact that CXCR3 is highly conserved in humans and rodents meant that serendipitously,

reagents targeting CXCR3 in humans generally show excellent activity against their rodent counterpart. This led to their considerable usage in animal models of disease. Since mice lacking CXCR3 were shown to tolerate mismatched cardiac allografts for extended periods [11], a proof of principle was established in which the efficacy of CXCR3 antagonists could be assessed *in vivo*. A procession of papers appeared in which the positive effects of small molecule CXCR3 blockade on allograft survival appeared in a variety of transplantation models [12-17]. Notably, some of these papers involved the broad spectrum antagonist TAK-779 which is better known for its activity at CCR5 [18] which therefore makes it difficult to assess the individual role of CXCR3 and its ligands in transplant rejection (discussed later). However, specific blockade of CXCR3 by the compound MRL-957 (structure undisclosed by Merck but covered in Patent WO 2007064553) resulted in only a moderate increase in graft survival. Moreover, independently-derived *Cxcr3* deficient mouse lines failed to reproduce the remarkable prolongation of cardiac graft survival observed in the original study [19]. Similarly, administration of the CXCR3 antagonist AMG 1237845 developed by Amgen (Figure 1) to allograft recipients prolonged allograft survival only modestly compared with control animals, with little differences in the numbers of $CD8^+$ or $CD4^+$ T-cells infiltrating the grafts observed in the presence or absence of treatment. Notably, there was some synergy observed when AMG 1237845 was dosed in conjunction with neutralizing anti-CD40L antibodies

Just one small molecule CXCR3 antagonist has entered clinical trials, namely the compound AMG 487 (Figure 1), a dihydroquinazoline analogue of AMG 1237845 which was originally discovered by scientists at Chemocentryx and subsequently

licensed to Amgen. AMG 487 was initially shown to selectively block CXCR3 *in vitro* with good potency ($IC_{50}$ of 8nM in a CXCL10 binding assay) and also possessed efficacy in a model of bleomycin-induced lung inflammation in mice when dosed daily at 3mg/kg [20]. In multiple dosing studies in humans (25mg of AMG 487), a complex pattern of dose-dependent and time-dependent pharmacokinetics emerged with AMG 487 being converted into two species; a pyridine-N-oxide metabolite with apparent four-fold increase in potency as deduced by *in vitro* cell migration assays and an *O*-deethylated metabolite with inhibitory properties at CYP2C9 and CYP3A [21]. Subsequent work by the authors uncovered an even more complex pathway for AMG 487 metabolism than first thought, involving the formation of hydroxylated metabolites of the initial *O*-deethylated metabolite which form adducts with GSH [22]. These complications led to efforts by Amgen to find superior molecules lacking major active metabolite formation which complicate clinical evaluation. Optimization of the quinazolinone series from which AMG 487 emerged identified a lead compound 34 which could block CXCR3-mediated migration *in vitro* at sub-nanomolar concentrations and significantly block leukocyte recruitment in the bleomycin-induced lung inflammation. Notably, doses 10-fold lower than the parental compound were effective [23]. Similarly, substitution at the C4-position of the azaquinazolinone core also yielded derivatives such as Compound 21 (Figure 1) with low nanomolar activity at CXCR3 correlating with a steady state plasma concentration of around 86 nM [24].

Against this back drop, Amgen pressed ahead with a phase II trial of AMD 487 in patients suffering with psoriasis. The rationale for the use of AMD 487 to treat this disease was supported by the fact that CXCR3 ligands are abundantly expressed in

psoriatic skin lesions [25] and that CXCR3 expression is observed on T cells infiltrating the lesion [26], suggestive of a role for the receptor:ligand axis in the disease process. It was therefore surprising that AMD 487 failed to show efficacy in psoriasis and the trial was terminated [27]. To date, AMD 487 remains the only CXCR3 antagonist to have entered clinical trials and given the enormous costs involved, it might be argued that its failure against a backdrop of general failure in targeting chemokine receptors in the inflammatory setting has deterred others from following suit. With the benefit of hindsight, the selection of psoriasis as an arena in which to test CXCR3 blockade is questionable. The supporting evidence has been dubbed to be from "guilt by association studies" [28], since by virtue of their expression, CXCR3 and its ligands were assumed to play a principal role in the disease. However, the precise role of CXCR3 in psoriasis is poorly understood. CXCR3 expression in psoriatic skin lesions has been reported to be also associated with mucosal mast cells, although the increase in staining seen was not statistically different from that of healthy controls [29]. Another study has implicated CXCL10 and CCL5 (a CCR1/CCR3/CCR5 ligand) in the recruitment of $CD56^{bright}$ $CD16^{-}$ NK cells (which make up almost 10% of the infiltrating cells in psoriatic lesions) and subsequent exacerbate inflammation [30]. Although psoriasis is thought to be a Th1-dominated disease, CCR4, CCR6 and their ligands CCL17 and CCL20 have also been shown to be upregulated in psoriatic skin [26, 31]. It is therefore plausible that in the absence of CXCR3, other chemokines can fulfill the role of T cell recruitment. The efficacy of CXCR3 blockade in an *in vivo* model of psoriasis may be informative in this respect.

## 2. Recently described small molecule antagonists of CXCR3

In the following years a growing number of small molecule CXCR3 antagonists have have been described in the literature. Scientists at Amgen followed up the discovery of AMG 487 with a series of 8-aza-quinazolinone analogues which avoided the pitfalls of AMG 487 with respect to metabolite activity and CYP3A4 inhibition. The most promising of these was Compound 25 (Figure 1) with an $IC_{50}$ of 45nM in *in vitro* chemotaxis assays with and a half-life of around 4 h in rodents [32]. In a bleomycin-induced model of mouse lung injury, Compound 25 dosed at 1mg/kg showed efficacy in inhibiting leukocyte migration to the lung, comparable to that observed in CXCR3 deficient mice.

Thoma and colleagues at Novartis described a series of ergolines, with the pick of these, named Compound 1 (subsequently renamed NIBR 2130, Figure 2) which possessed low nanomolar activity in ligand binding and calcium flux assays using CXCR3 transfectants. It also possessed excellent selectivity, with no discernable activity at serotonin, dopamine and adrenergic receptors, despite obvious structural similarities to LSD [33]. Initial SAR studies by the group determined that methylation of the urea group or replacement with carbamate resulted in a loss of activity. Likewise, methylation of the indole nitrogen or the addition of a bulky benzyl group adversely affected potency. In contrast, methyl-substitution of the phenyl ring or its replacement with a cyclohexyl group was tolerated, suggesting that the unsubstituted phenylurea core was required for optimal activity. Subsequent derivation of a series of amides resulted in the identification of optimized compounds, notably Compound 11a (Figure 2) with an $IC_{50}$ of 5nM in both ligand binding and calcium flux assays and favourable PK properties in rats [34].

In 2010, the group of Amanda Proudfoot at Merck Serono identified a lead compound via a HTS of CCR3 transfectants assaying cAMP levels in response to ligand, taking advantage of the propensity of CXCR3 to couple to to Gαi proteins. SAR work revealed a preference for lipophilic groups on the amide N-substituent whilst substitutions on the sulfonamide ring were poorly tolerated, leading to the discovery of Compound 27 with an $IC_{50}$ of 13 nM in chemotaxis assays. Unfortunately, Compound 27 suffered from an adverse PK profile and was not progressed [35]. Scientists at Ligand Pharmaceuticals (formerly Pharmacopeia) in collaboration with Merck identified a novel series of CXCR3 specific molecules based around a lead piperazinyl-piperidine pharmacophore flanked on either side by a nicotinamide and 4-chlorobenzyl group. The molecule had moderate antagonist activity in ligand binding assays [36]. Subsequent SAR work highlighted an absolute requirement for the pyridinyl piperazinyl-piperidine core and the nicotinyl amide linkage. A preference for para-substituted benzyl groups on the piperidine moiety and methyl substitution on the piperazine ring was noted. A subsequent report of further SAR studies reported that changes to the 2-position of the piperazine increased the affinity of the antagonist for CXCR3 (notably increasing the size of the alkyl group at the 2-position of the piperazine ring) and resulted in the discovery of Compound 18j (Figure 2) with 20-fold improved affinity for hCXCR3 and subnanomolar activity at mouse CXCR3 [37].

Further SAR work with a 6-amino-3-chloropyrazine scaffold was undertaken with the aim of to improving the hERG profile of Compound 18j. This involved exploration of the polar substituents of the pyrazine amide and the addition of polar groups to the piperidine fragment. Ultimately this lead to the discovery of Compound 16e (Figure

2) with an improved PK profile and reduced hERG activity but still retaining single-digit affinity for CXCR3 affinity [38]. Additional SAR was then carried out to replace the amide group with heterocycle surrogates, the objective being to avoid metabolic cleavage of the left-hand side amide moiety but maintain identical or improved biological profiles [39]. Oxadiazole was found to be the most promising heterocycle in terms of maintaining CXCR3 binding activity. Subsequent synthesis of substituted oxadiazoles in an effort to replace the pyridine in the core of the molecule identified the primary amide analog 2a (SCH 546738, Figure 2) which was elected for *in vivo* proof-of-principle studies [39]. Subsequent dosing of SCH 546738 in mice at 40 mg/kg showed it to have significant efficacy in a collagen-induced arthritis model. Efficacy at a variety of doses was also shown in rat and mouse models of experimental autoimmune encephalomyelitis and rat cardiac allograft rejection [40].

## 3. Targeting CXCR3 in "non-traditional" disease areas

A number of papers have been published in recent years describing potential roles for the CXCR3 axis in disease areas beyond the traditional Th1 arena. This may lend credence to the belief that an effective, well-tolerated CXCR3 antagonist could be of virtue provided that it is used in the correct clinical setting. One such setting is cancer, where chemokines and their receptors have long been implicated in tumour metastasis [41]. Cambien and colleagues recently showed that CXCR3 was overexpressed in biopsies taken from colorectal cancer (CRC) patients. Intravenous injection into mice of a $CXCR3^+$ CRC cell line resulted in cells homing to the lung and and subsequent tumour development, with tumour growth sensitive to subcutaneous injections of AMG 487 (5 mg/kg) [42]. The same group also showed in a subsequent study that identical doses of AMG 487 were effective in blocking metastasis of the

K7M2 osteosarcoma cell line to mouse lungs [43]. Both studies suggest that CXCR3 and its ligands support the initial homing of cells to the lung and subsequent growth and survival, although it should be cautioned that in the first study, metastasis to the liver was unaffected by AMG487 treatment suggesting that pathways for migration and survival may be be highly tissue dependent [42].

A role for CXCR3 in neuronal signaling has been suggested in a couple of studies in rodents. Both CXCR3 and CXCL10 were was shown by Qu et al to be upregulated in the dorsal root ganglion in a mouse model of allergic contact dermatitis following application of the sensitizer dibutylester to the skin. Blockade of CXCR3 signaling by *s.c.* injection of AMG 487 resulted in an inhibition of itch-related behaviours such as biting of the site of application, suggesting that the CXCL10/CXCR3 axis may play a role in the pathology of chronic itch as seen in contact hypersensitivity [44]. Metastasis of mammary tumours to the patients' bones is often accompanied by pain. Using a rat model of metastatic breast cancer, Bu and colleagues showed that expression of CXCR3 and CXCL10 was upregulated in the spinal cord during metastasis. Moreover, and the associated pain (as measured by mechanical allodynia) and the activation of microglia within the spinal cord was reversed by intrathecal injection of AMG 487 [45].

Infiltration of the pancreatic islets by lymphocytes is increased during the early stages of pancreatitis, which is thought to be an initiating event in type 1 diabetes (T1D) and is associated with increased expression of CXCL10 in newly diagnosed patients [46]. Consequently, CXCR3 has attracted attention as a therapeutic avenue, although its relevance is controversial. In a streptozotocin-induced model of T1D in

rats, He and colleagues reported that the elevated serum levels of CXCL10 observed in a saline treated group could be reduced by *s.c.* injection of NBI-74330 (Figure 3) at a daily dose of 2μg/kg which correlated with significantly reduced apoptosis of islet cells [47]. In contrast, in a virally-induced mouse model of diabetes. Christen et al showed the CXCR3 antagonist NIBR2130 (Figure 2) to have little effect upon glucose tolerance [48], although direct blockade of IP-10 by the same group in the same model was previously reported to be efficacious [49]. This may suggest that another receptor can mediate the effects of CXCL10, indeed TLR4 has been proposed by to function as a CXCL10 receptor on human pancreatic islet cells as determined by pull-down assays with biotinylated CXCL10 [46].

An intriguing role for CXCR3 in the pathogenesis of Alzheimer's disease has recently been put forward by Krauthausen et al. In a APP/PS1 transgenic mouse model of the disease, CXCR3 deficiency was associated with reduced plaque deposition in the hippocampus and reduced serum levels of amyloid-β [50]. Studies of CXCR3 sufficient mice showed cerebral levels of CXCL10 to be significantly elevated during disease compared with CXCR3 deficient littermates which was associated with reduced microglial activation, notably TNFα generation. This could be blocked *in vitro*, by the application of which could be blocked *in vitro* by the CXCR3 antagonist 12o (Figure 3) [51]. Moreover, CXCR3 deficiency protected the mice from learning and memory dysfunction as measured by spatial trials such as the Morris water-maze. Before studies in humans are contemplated, it remains to be seen if antagonism of CXCR3 is also effective in this model with one potential pitfall being the ability of molecules such as 12o to cross the blood-brain barrier.

As CXCR3 and its ligands are indicative of Th1repsonses, it is perhaps unsurprising

that increases in the levels of CXCR3 ligands are often associated with viral infections. Elevated serum levels of CXCL10 have been reported to be associated with hepatitis C virus (HCV) infection, notably in those chronically infected and/or failing to respond to IFN-α therapy [52, 53]. The apparent contradiction as to why elevated levels of a $CD8^+$ T cell-recruiting chemokine are associated with failure to respond to therapy can be explained by the fact that CXCL10 undergoes N-terminal cleavage by the enzyme dipeptidyl peptidase IV (DPP IV) resulting in the generation of a potent and efficacious CXCR3 antagonist [54]. Casrouge and colleagues showed that chronic HCV infection is associated with increased circulating levels of DPP IV suggesting that CXCR3 blockade is associated with treatment failure [55]. Sadly, a phase I trial of the DPP IV inhibitor sitagliptin with the aim of testing this hypothesis was terminated due to adverse events [56].

**Conclusions**

While we have come a very long way since the description of the first small molecule CXCR3 antagonist, to date, only AMG 487 has made it into the clinic, with disappointing results. Failure is true not only of AMG 487 but of chemokine receptor antagonists in general and the field of drug discovery as a whole. Few molecules successfully make it all the way from the laboratory bench to the bedside. The reasons for failure are probably many and varied [57-59] but a key problem is proving beyond reasonable doubt that the target is critical for disease pathogenesis and that effective blockade will be therapeutic.

The small molecule antagonist TAK-779 (Figure 3) is often described in the literature as a CCR5/CXCR3 antagonist, albeit with different potencies at the two receptors in

chemotaxis assays (respective $IC_{50}$ values of 1.9 nM and 15.8 μM [60, 61]). It also has considerable activity at CCR2 ($IC_{50}$ of 5.8 nM, [60]), thus when dosed correctly, it takes three receptors out of the clinical equation. It is noteworthy that in several *in vivo* studies TAK-779 showed significant efficacy. These included disease models as diverse as collagen-induced arthritis [62], ischaemia reperfusion injury [63, 64], colitis [65], transplantation [12, 16], experimental autoimmune encephalomyelitis [66], prostatitis [67] and cardiac allograft vasculopathy [68]. This might suggest that deliberately targeting more than one receptor may be the way forward in some disease scenarios. Sadly for such a potential panacea, TAK-779 suffers from poor oral bioavailability. SAR work was undertaken in which TAK-779 underwent ring expansion of (6,7)-fused nuclei to (6,8)-fused nuclei, had its quaternary ammonium moiety replaced with a polar sulfoxide moiety and had its methyl group substituted with a 4-(2-butoxyethoxy) group, leading to the next generation antagonist TAK-652 (Figure 3) [69]. Unfortunately, this SAR work resulted in a loss of activity at CXCR3 [61]. Site-directed mutagenesis aimed at elucidating the TAK-779 binding site within CXCR3 was inconclusive, whilst pinpointing key receptor residues that contacted AMG 487 [61]. A homology modelling approach may be informative and it is anticipated that the availability of crystal structures for CCR5 with docked antagonists may prove useful in this respect [70] as might the incorporation of mutations such as 3.35A, N3.35S, and T2.56P which lead to constitutively active CXCR3 and at which molecules such as AMG 487 act as inverse agonists [71].

An alternative approach to targeting CXCR3 via physical blockade is to actively induce its desensitization by treatment with an agonist, mimicking the approach taken with superagonists of gonadotrophin releasing hormone (GnRH) receptor, so

called “chemical castration”. Stroke and colleagues at Pharmacopeia first described two distinct chemical classes of small molecule CXCR3 which contained a positively charged amino acid component [72]. One of these agonists, PS 372424 (Figure 4), was subsequently shown to inhibit T-cell recruitment in response to rheumatoid arthritis synovial fluid in a humanized SCID mouse model [73]. PS 372424 appears to be a full agonist in several *in vitro* assays [74] and functions by mimicking a key region of CXCL10 as deduced by mutagenesis [75]. The group of Rob Leurs at VU University have recently described the first small molecule non-peptide agonists of CXCR3, the bi-aryl type ligands VUF11222 and VUF11418 (Figure 4) [76]. An initial ligand binding screen of a small compound library identified a lead adamantane-guanidine molecule, able to displace more than 50% of radiolabeled CXCL10 from CXCR3 transfectants when employed at a concentration of 10μM. Replacement of the guanidine group by a small quaternary ammonium group and the addition of a substituted aryl ring to the benzyl group, led to the identification of several bis-aryl-based ligands with affinity for CXCR3 in the region of 100nM [77]. Subsequent SAR work involved the introduction of halogen atoms to the outer aryl ring, generating a series of small molecule CXCR3 agonists in GTPγS binding assays with a broad range of potiencies and effiacies. In contrast, modification of the bicycloaliphatic group had limited effect on affinity for CXCR3 or agonist activity [76]. To date, no data has been published regarding the efficacy of either VUF11222 or VUF11418 *in vitro.*

## 4. Expert Opinion

Despite the discovery of several diverse classes of antagonists with low or even sub nanomolar affinity for CXCR3 and clear evidence of their *in vivo* efficacy, are we any

closer to progressing such molecules to the clinic? Clinical trials are of course extremely expensive and the failure of one molecule after another in the field does not build confidence. I surmise that as Amgen “sneezed” with the failure of AMG 487 in the clinic, the remaining players “caught a cold” and attempts to progress CXCR3 in the clinical setting slowly ground to a halt. Reading between the lines, the descriptions of CXCR3 antagonists in the literature these last 5 years is suggestive of CXCR3 programs being wound down and molecules being dusted down and placed on the shelf. Indeed, an online search of the current clinical trials in almost 200 countries (https://clinicaltrials.gov) suggests there are currently no molecules targeting CXCR3 in clinical trials. However, from a nadir in 2012, when no patents regarding CXCR3 antagonists were filed worldwide, things have perhaps begun to pick up. A handful of patents have been filed in recent years by several companies. These include Galderma, Daiichi Sankyo, Sanofi and Actelion. Data concerning these molecules is currently scarce, although the molecules are briefly described in a recent review by Andrews and Cox [78].

What fate awaits this recently patented compounds? If we are to learn anything from the successes of targeting chemokine receptors in human disease, it is that when the target is proven beyond doubt to be critical for pathogenesis, a decent small molecule antagonist stands a chance of success. For example, targeting overactive CXCR4 in WHIM syndrome with plerixafor [79] or blocking CCR5 as major portal for HIV-1 entry [80] with maraviroc met with clinical approval and some degree of success.

For CXCR3 antagonist programs, what is needed then is clear clinical evidence that targeting CXCR3 in one disease setting or another is a valid approach. For example, despite its success in HIV-1 treatment, maraviroc has failed in a phase IIa study in rheumatoid arthritis patients, highlighting the fact that target validation is of critical importance. [81]. In addition, phase I data showing the most promising CXCR3 antagonists are well tolerated and achieve sufficient levels of blockade is required. Such an approach requires ongoing collaborations between academia (target validation) and industry (antagonist development). An attention to detail by basic scientists and clinicians may also highlight alternative avenues for treatment with CXCR3 antagonists. For example, the CXCR4 antagonist plerixafor was originally developed as an HIV-1 treatment, but subsequently found use as a stem cell mobilizing agent and a WHIM syndrome treatment.

The complex pharmacology of CXCR3 [8] suggests that it might also provide routes for pharmacologists and medicinal chemists to tease apart biased signaling at the receptor. It may well be that particular signaling pathways (G protein versus β-arrestin) are more rooted in one disease scenario than another and that specific targeting of one pathway may be more beneficial and perhaps better tolerated. A group at Friedrich Alexander University recently described a boronic acid-based CXCR3 antagonist which selectively inhibited β-arrestin 2 recruitment, but spared G protein activation [82]. The same group also reported a novel compound that did the reverse, selectively inhibiting G protein activation over β-arrestin 2 recruitment [83]. Such molecules may provide a more tailored approach to the blockade of CXCR3 signalling and their progress is eagerly anticipated.

**Article highlights**

- CXCR3 is implicated in several clinically important diseases, yet the sole trial of a CXCR3 antagonist to-date met with failure. Potential reasons for this are discussed.

- Despite this failure, several CXCR3 antagonists of different classes have shown efficacy in animal models of disease, giving cause for optimism.

- Some of these *in vivo* studies lie outside of the traditional “Th1” area suggesting new disease avenues for successful CXCR3 antagonists.

- Several novel CXCR3 antagonists with a variety of pharmacophores have recently described in the literature and are discussed.

- The complex pharmacology of CXCR3 may provide opportunities for a biased approach to antagonism with the potential for greater selectivity/efficacy.

This box summarizes key points contained in the article.

## Figure Legends

**Figure 1.** The chemical structures of CXCR3 antagonists developed by Amgen/Chemocentryx.

**Figure 2.** The chemical structures of CXCR3 antagonists developed by Novartis (NIBR 2130 and Compound 11a), Merck Serono (Compound 27) and Ligand Pharmaceuticals/Merck Research Labs (Compound 18j, Compound 16e and SCH-546738).

**Figure 3.** The chemical structures of the specific CXCR3 antagonist NBI-74330 developed by Neurocrine Biosciences and the broad spectrum CCR5, CCR2 and CXCR3 antagonist TAK-779, and CCR2/CCR5 antagonist TAK-652 first reported by Takeda.

**Figure 4.** The chemical structures of the CXCR3 antagonists PS 37242 developed by Ligand Pharmaceuticals (formerly Pharmacopeia) and VUF 11222 and VUF 11418 discovered at the Vrije Universiteit, Amsterdam.

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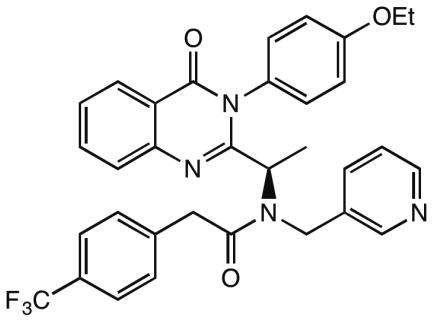
These last two papers from the group of Nuska Tschammer show that it is possible to generate biased small molecule CXCR3 antagonists that selectively inhibit different signalling pathways downstream of CXCR3 activation.

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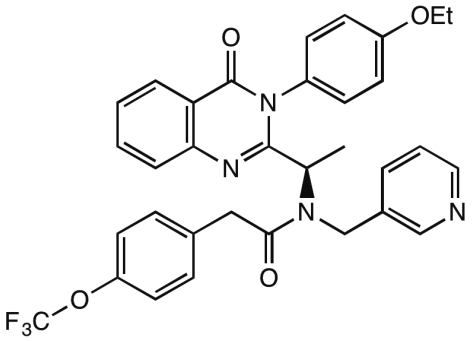


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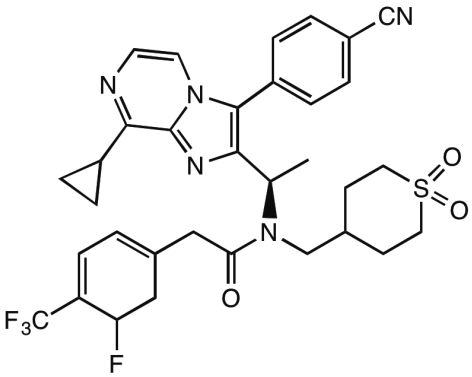


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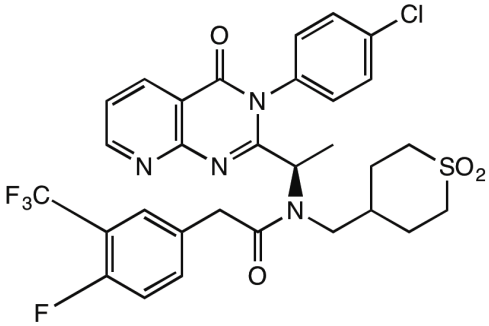


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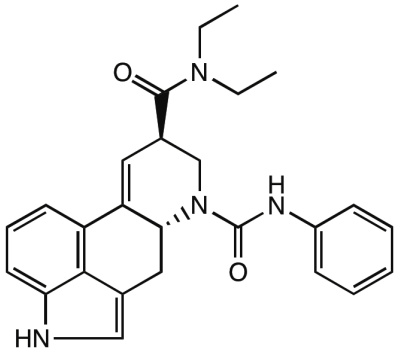
Figure 1



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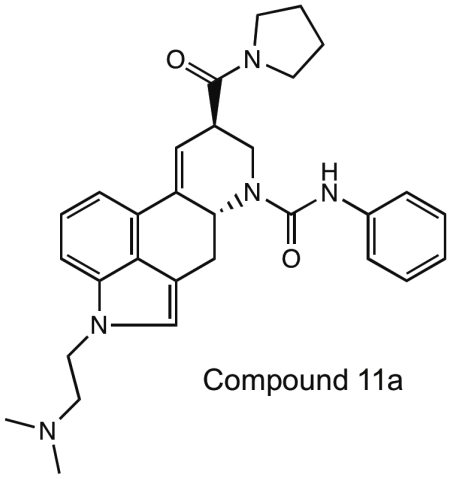

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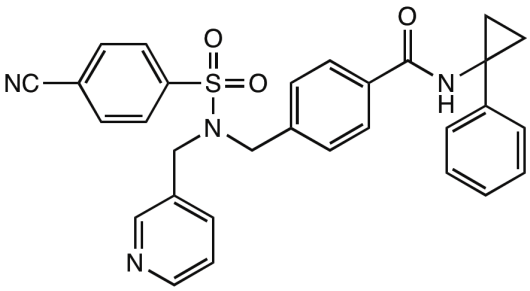

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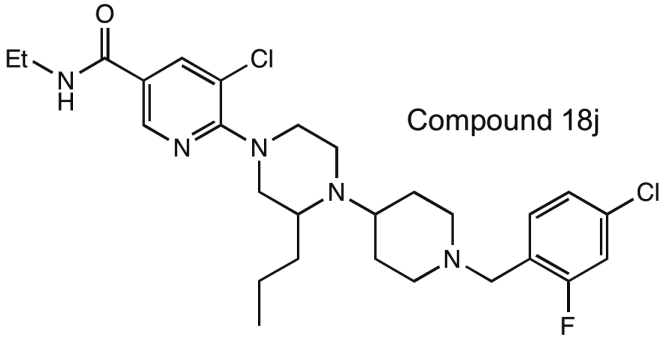

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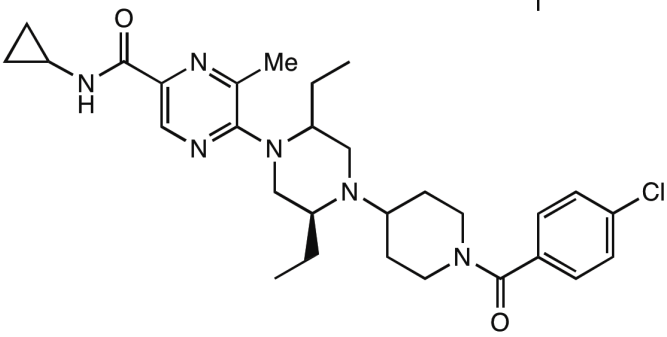

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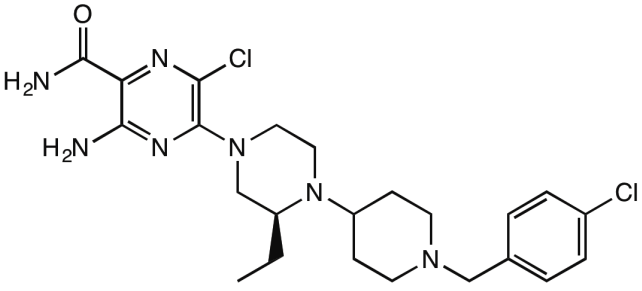

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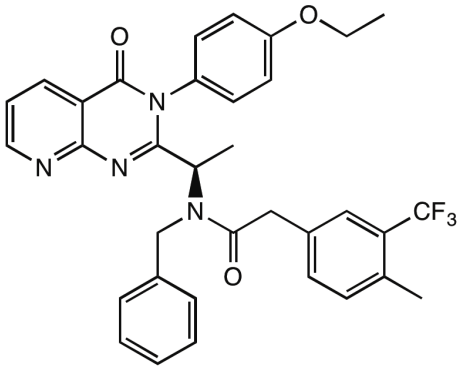
Figure 2



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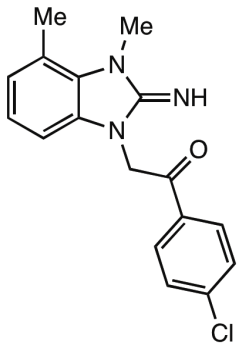

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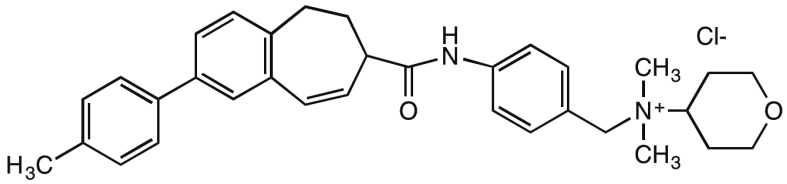

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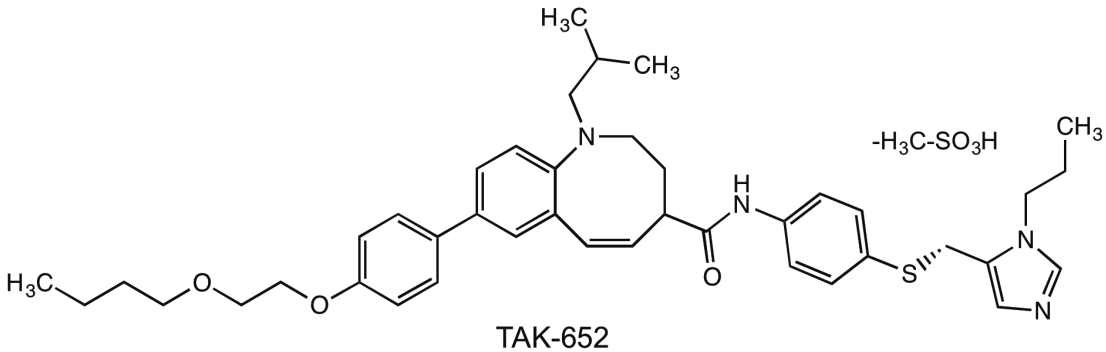

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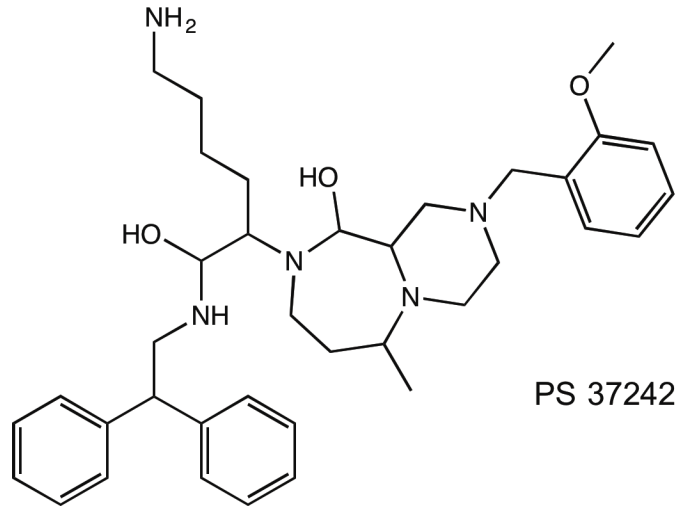
Figure 3

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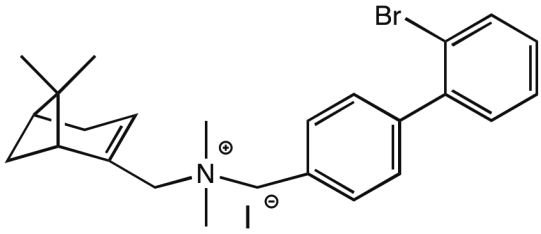


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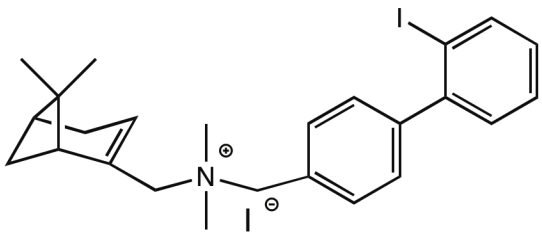


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Figure 4