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CCR5 receptor antagonists in preclinical to phase II clinical development for treatment of HIV

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Abstract

Introduction—The chemokine receptor CCR5 has garnered significant attention in recent years as a target to treat HIV infection largely due to the approval and success of the drug Maraviroc. The side effects and inefficiencies with other first generation agents led to failed clinical trials, prompting the development of newer CCR5 antagonists.

Areas covered—This review aims to survey the current status of ‘next generation’ CCR5 antagonists in the preclinical pipeline with an emphasis on emerging agents for the treatment of HIV infection. These efforts have culminated in the identification of advanced second-generation agents to reach the clinic and the dual CCR5/CCR2 antagonist Cenicriviroc as the most advanced currently in phase II clinical studies.

Expert opinion—The clinical success of CCR5 inhibitors for treatment of HIV infection has rested largely on studies of Maraviroc and a second-generation dual CCR5/CCR2 antagonist Cenicriviroc. Although research efforts identified several promising preclinical candidates, these were dropped during early clinical studies. Despite patient access to Maraviroc, there is insufficient enthusiasm surrounding its use as front-line therapy for treatment of HIV. The non-HIV infection related development activities for Maraviroc and Cenicriviroc may help drive future interests.

Keywords

CCR5; CCR2; CXCR4; Maraviroc; HIV; entry inhibitors; antagonists; Cenicriviroc; chemokine receptors

1. Introduction

Since its discovery in 1983, the human immunodeficiency virus (HIV-1) has eluded broad-spectrum vaccination efforts and remains an imminent threat to public health with 35 million

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Declaration of interest

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infections worldwide [1]. The development of highly active antiretroviral therapy (HAART) has significantly curbed the mortality rate previously associated with HIV-1 and has transformed the diagnosis from a death sentence to a chronic, manageable condition for many [2,3]. HAART consists of a personalized regimen of three or more antiviral drugs that interfere with various aspects of the viral lifecycle. To date, over 25 agents have been clinically approved for the treatment of HIV-1; however, the development of new antiviral agents is driven by the acquisition of drug resistance to preexisting regimens, simplified dosing schedules, and reduced pill burden to enhance patient compliance [4]. The classes of HAART medicines include both nucleoside and non-nucleoside reverse-transcriptase inhibitors (NNRTIs), HIV-1 protease inhibitors (PIs), HIV-1 integrase inhibitors, and viral–host entry inhibitors [5]. At the time of this writing, Enfuvirtide (Fuzeon[®], Genetech CA, USA) and Maraviroc (MVC) are the only two clinically approved antiviral drugs that block HIV-1 entry [6]. Fuzeon[®] is an injectable fusion inhibitor that binds gp41 and prevents its insertion into the plasma membrane, but this drug is inconvenient to administer and produces significant inflammation at the injection site that eventually causes patient noncompliance precluding further use. MVC is an orally administered, potent CCR5 antagonist that prevents the HIV-1 envelope protein gp120 from binding to the host-based chemokine and CD4 receptor, making this the only mammalian-host target-based HIV medication (*all references made to HIV in this review will refer to the HIV-1 form of the virus*).

2. CCR5 biology and chemokine receptor-based HIV tropism

CCR5 is a promiscuous seven-transmembrane G-Protein Coupled Receptor (GPCR) that binds several endogenous chemokines to mediate receptor activation [7]. Macrophage inflammatory protein 1 α (MIP-1 α , CCL3) and 1 β (MIP-1 β , CCL4) and Regulated on Activation, Normal T cell Expressed and Secreted (RANTES) (CCL5) are potent agonists, whereas CCL8 and CCL13 are partial agonists that exhibit a spectrum of CCR5 activation. CCL7 has also been identified as a ligand for CCR5; however, CCL7 does not appear to induce receptor activation and is believed to be a natural antagonist [7]. Binding of CCR5 to an agonistic chemokine triggers a signaling cascade via intracellular G-coupled proteins located at the C-terminus to coordinate leukocyte trafficking [8,9] and recruit immune effector cells to inflamed or damaged tissues to prime the adaptive immune response [10]. The inhibition of CCR5 with MVC significantly reduces T-cell migration in peripheral blood mononuclear cells (PBMCs) in a dose-dependent manner and arrests T-cell proliferation at higher concentrations (100 μ M) *in vitro* [11]. Similar reductions in chemotaxis were also noted for monocytes, macrophages, and monocyte-derived dendritic cells toward their cognate ligands in the presence of MVC (0.1–10 μ M) [12]. These intrinsic immunomodulatory effects associated with CCR5 antagonism are highly desirable for the treatment of inflammatory and autoimmune conditions including graft-versus-host disease [13], multiple sclerosis [14], and ischemic stroke [15]. However, CCR5 has also been heavily implicated in the development of CD8⁺ T cells [16–18] which play a critical role in the clearance of virus-infected cells and in the pathogenesis of HIV infection [19,20]. Interestingly, the introduction of deleterious mutations or deletions within the CCR5 gene does not appear to induce dire immunological consequences beyond predisposition to West

Nile virus [21,22], tickborne encephalitis [23], *Toxoplasma gondii* [24], and a handful of other central nervous system-localized infections [25,26].

CCR5 is intimately involved in HIV entry and renders several immune cells permissible to infection including memory/effector T cells, dendritic cells, macrophages, monocytes, B cells, and natural killer cells [27–29]. Entry is initiated when the viral envelope protein gp120 binds to CD4 on the target cell surface and subsequently anchors itself to a co-receptor, either CCR5 or CXCR4, which is essential for virion fusion to the plasma membrane. Inhibition of CCR5 and CXCR4 alone is sufficient to prevent HIV entry. Viral strains that bind CCR5 are classified as R5-tropic (R5), strains that interact with CXCR4 are X4-tropic (X4), and dual/mixed tropic viruses use either CCR5 or CXCR4 to mediate entry [30]. This is problematic for CCR5 and/or CXCR4 antagonists in that inhibition of one chemokine may cause a tropism shift, potentially exacerbating disease progression. Several lines of evidence have linked X4-tropic HIV with pronounced T-cell depletion, immunosuppression, and rapid progression to AIDS [31]. In contrast, CCR5-tropic virions generally produce a relatively mild asymptomatic infection over several years before compromising the host. One may speculate that inhibition of CXCR4 would lead to a less pathogenic infection; however, there is a clear preference for HIV to use CCR5 over CXCR4, rendering CCR5 as the more desirable drug target.

A natural 32-base pair deletion within the CCR5 gene (CCR5 Δ 32) has been extensively documented in 10–20% of the Caucasian population that confers resistance to HIV [32,33].

Δ 32 heterozygotes express both functional and truncated CCR5 which confers a certain degree of protection from R5-HIV; however, these individuals are still permissive to infection [34]. Only individuals who express both CCR5 Δ 32 alleles (1% to 2% of the Caucasian population) completely lack surface expression of CCR5 and are immune to R5 HIV [35]. The curative potential of permanent CCR5 deficiency in HIV-infected persons was realized in two landmark studies demonstrating the first and only reported sterilizing cure for HIV infection following a stem-cell transplant of homozygous CCR5 Δ 32/CCR5 Δ 32 donor progenitor cells to an HIV-positive patient suffering from acute myeloid leukemia [36,37]. As of 2016, the so-called ‘Berlin patient’ remains HIV-free in the absence of antiretroviral therapy with no signs of disease progression or viral rebound. However, this procedure failed to cure a 27-year-old HIV-positive patient suffering from T-cell lymphoma and led to a shift in viral tropism (CCR5 to CXCR4) that resulted in death [38]. These findings combined with the low prevalence of homozygous CCR5 Δ 32 donor stem cells and a significant mortality (40–55%) [39,40] make stem-cell transplantation ill-suited for widespread clinical applications and encourages pharmaceutical intervention through a CCR5-based HAART-type regimen.

3. First-generation CCR5 antagonists

The area covering the potential of first-generation CCR5 antagonists for HIV treatment was previously reviewed before all clinical outcomes were known. Since then, MVC (1, Figure 1), an orally administered and potent CCR5 antagonist, received Food and Drug Administration (FDA) approval in 2007. This drug demonstrates a long plasma half-life ($t_{1/2}$ 15–23 h) [41] to support a convenient once-a-day dosing schedule [42,43] and exhibits

broad-spectrum antiviral activity across several R5 isolates including 200 clinically derived HIV envelope pseudo-viruses with an average IC₉₀ of approximately 14 nM [21]. Data collected from clinical trials (MOTIVATE (Maraviroc Therapy in Antiretroviral Treatment-Experienced HIV-1-Infected Patients) 1 and 2) indicate that MVC is generally well tolerated and significantly repressed viral load in R5-infected HAART-treatment-experienced patients; however, MVC is also hepatotoxic and is not recommended for treatment-naïve patients or individuals infected with X4- or dual-tropic R5X4 virus [44–46]. The M/R5-tropic requirement of prequalification for MVC administration led the drug sponsor to develop a suitable test to determine viral population tropism within patients. This test, which now is known under the name TROFILE (Monogram/Quest Biosciences, San Francisco, CA) is currently used by clinicians [47]. MVC is the only tropism-dependent HIV drug and is designated as a backup regimen partly due to this prescreening requirement. Although this test is not approved by the FDA, it is required to ensure R5-only tropism and drug treatment success. Adoption of this practice was supported in the clinical trials of MVC, where a first-generation version of TROFILE test proved to be inaccurate allowing patients with mixed R5/X4 virus populations to take MVC. Significant drug failure was observed in these individuals [48]. The CCR5 to CXCR4 tropism shifts in patients taking MVC is currently unknown; however, observations of tropism shifts toward X4 using virus have been observed in naïve and HAART (non-MVC)-treated individuals. Shifts also occur naturally during viral evolution as the X4-tropic virus is the main driver in the development of AIDS [49,50]. Thus, the tropism test is both a prequalifier as well as a limitation to CCR5 antagonists in the HIV setting since no other HIV medication requires this pretest. However, it is worth noting that current tropism assays have increased sensitivity to reliably detect X4-tropic HIV with rapid turnaround and at a low cost.

Despite the need for the viral tropism test and initial setbacks involving implementation, there have been several clinically significant developments involving MVC showing its adoption into the mainstream of HIV treatments with some unexpected benefits [51]. Adding MVC to an antiviral regimen consisting of raltegravir/tenofovir/emtricitabine resulted in faster reduction of 2-LTR⁺ (2-long term repeat) newly infected cells and recovery of CD4⁺ T-cell counts and a modest reduction in total reservoir size after 48 weeks of treatment [52]. In another study, MVC was compared with efavirenz both in combination with zidovudine/lamivudine (MERIT [Maraviroc versus Efavirenz in Treatment-Naïve Patients] trial) in HIV-infected patients, as it has been demonstrated that patients on HAART therapy are at increased risk for developing metabolic abnormalities that include elevated levels of serum triglycerides, low-density lipoprotein cholesterol (LDL-c), and reduced levels of high-density lipoprotein cholesterol [53]. In this study, MVC was not associated with elevations in total cholesterol, LDL-c, or triglycerides and showed beneficial effects on lipid profiles of dyslipidemic patients [54].

MVC has also been evaluated as a preexposure prophylaxis agent alone and in combination with reverse-transcriptase inhibitors. Preliminary studies with macaques have demonstrated that oral administration of MVC (44 mg/kg) alone is insufficient to prevent HIV infection despite high drug concentration in rectal tissues 24 h prior to inoculation [55]. Nonetheless, MVC has been pushed to human trials in the NEXT-PrEP (Novel Exploration of

Therapeutics for PreExposure Prophylaxis) study although no data on the outcome of the study are available at the time of this writing [56].

In 2001, scientists at Schering-Plough (Kenilworth, NJ) identified the clinical candidate Vicriviroc (**2**, VVC, Figure 1) as a noncompetitive allosteric CCR5 antagonist [57,58]. VVC reached phase II trials for treatment-naïve patients; however, these efforts were halted after patients experienced viral rebound with continued treatment [59]. Subsequent studies in HIV-infected treatment-experienced patients revealed that VVC demonstrated potent virologic suppression; however, this was accompanied by an increase in liver malignancies that further stalled the development of VVC [60]. Around the same time, GSK's (Brentford, UK) CCR5 program identified spirodike-topiperazine derivative Aplaviroc (AVC) (AK-602 and GSK-873140). AVC (**3**, Figure 1) exhibited excellent activity against HIV-1_{Ba-L} with an IC₅₀ value of 0.4 nM and demonstrated a favorable pharmacokinetic profile in monkeys with an oral bioavailability of 30% [61]. Unfortunately, phase II clinical trials were terminated due to severe hepatotoxicity observed in infected patients.

The success of MVC and the failures of VVC and AVC have recently been scrutinized in reference to lack of druglikeness in these compounds as the potential reasons for failure in the clinic and setting the stage for future innovation [62]. Furthermore, the side effects and insufficiencies associated with VVC and AVC versus the excellent profile of MVC became the focus of ensuing research that would expand to a number of follow-ups and include compounds with activity against a second chemokine as a desirable approach. This review aims to survey the current status of 'next-generation' CCR5 antagonists in the early-stage pipeline with an emphasis on emerging agents and strategies as treatments for HIV infection.

4. Second-generation small-molecule CCR5 antagonists

The design of new CCR5 antagonists involves a number of different strategies including *de novo* drug design, high-throughput virtual screening, scaffold hopping, and other unique approaches such as drug-antibody conjugates. Many of the medicinal chemistry efforts have modified the scaffolds of MVC, VVC, and AVC to acquire novelty, increase potency, or enhance ADME (Absorption/Distribution/Metabolism/Excretion) properties. Few candidates reached early preclinical/clinical evaluation, and some were found to be superior to MVC.

In vitro bioassays are the first line of assessment for the activity of small-molecule CCR5 antagonists. The RANTES-binding assay utilizes radiolabeled chemokines of CCR5 such as [¹²⁵I]-RANTES, [¹²⁵I]-MIP-1 α , and [¹²⁵I]-MIP-1 β to measure the competitive displacement of the radiolabeled ligand by the small molecule at the receptor site. In terms of chemokine receptor functional response, the calcium mobilization assay can be used to determine whether HIV has engaged with the co-receptor CCR5 or CXCR4, which would trigger a measurable flux of intracellular calcium. These assays can be used to measure responses to all relevant chemokine receptors including CCR5, CCR2, and CXCR4.

A second pivotal assay for small-molecule assessments is the anti-HIV assay, otherwise described as an anti-infectivity or antiviral assay, which is the main measure of the small molecule's capacity to inhibit the replication of HIV in cells. Often PBMCs are the medium

in which the assay is conducted as HIV primarily targets these cells, but often a more specific cell line such as peripheral blood lymphocyte (PBL) cells can be used. Inhibition of viral replication is measured directly by amount of viral RNA transcription or reverse-transcriptase enzyme activity. The IC_{50} values extrapolate directly to the clinical efficacy to suppress HIV infection and replication. These assays are not mechanistically sensitive to the type of antiviral activity and are used to measure activity against a multitude of different HIV targets including cell entry, reverse transcriptase, protease, and integrase.

Several surrogates to the PBM/LC (Peripheral Blood Mononuclear/Lymphocyte Cells) assays can also be used to detect viral infection and offer advantages of mechanistic bias or altered biochemical readouts. The CCR5 fusion assay offers a mechanistic bias for entry inhibitors in that it measures the ability to block the HIV entry by preventing the binding of gp120 or gp160:CD4 complex to the co-receptor CCR5. Another assay that can be useful in the initial characterization of anti-HIV compounds is the reporter gene-based multinuclear activation of a beta-galactosidase indicator (MAGI) assay usually conducted in receptor-transfected CEM (Human T-lymphoblastoid Leukemia) or human osteosarcoma (HOS) cells.

Promising preclinical candidates were met with a potency criterion in either one or several of the *in vitro* assays at low nanomolar levels of inhibition. However, their poor pharmacokinetic profile in animal studies, unsafe levels of inhibition to the human ether-à-go-go-related gene (hERG), or inhibition of the metabolic enzyme CYP2D6 halted their advancement into the clinic. Newer scaffolds identified from alternative techniques lie at a more nascent stage. The most advanced approach seems to involve incorporating dual chemokine activity. We will describe these efforts in this section.

4.1. Piperidine amide compounds

4.1.1. MVC-inspired/tropane second-generation compounds—In 2011, Pfizer (New York, NY) reported a second-generation oral CCR5 antagonist imidazopiperidine drug candidate PF-232798 (**4**, Figure 2) during a follow-up program with the intention of improving the oral absorption of MVC [63]. Twenty-two compounds were synthesized, and lead candidate PF-232798 was identified after an *in vivo* pharmacokinetic screening in both rats and dogs. PF-232798 demonstrated potent anti-HIV activity ($IC_{50} = 2.0$ nM, HIV-1_{Ba-L}) and modest hERG activity ($IC_{50} = 12$ μ M) that was comparable to MVC and exhibited substantial oral absorption in rats and dogs. Stability studies in human liver microsomes indicated that PF-232798 was metabolically stable and not a suitable substrate for CYP3A4, which could translate to a reduction in dosing frequency and a prolonged half-life. In addition, the compound retained antiviral activity against B-clade MVC-resistant HIV-1 isolate strain CC185. Phase II clinical trials were completed in August of 2013 with no adverse safety reactions reported at levels up to 250 mg; however, no other information about the status of PF-232798 (**4**) has been reported [64].

GSK 163929 (**5**) is a 4,4-disubstituted piperidine scaffold that was discovered at GlaxoSmithKline through a modular, high-throughput chemistry approach [65]. GSK 163929 had good potency with IC_{50} values of 4.26 and 3.47 nM against HIV-1_{Ba-L}-infected HOS and PBL cells, respectively [66]. Excellent pharmacokinetic properties were observed,

and a 7-day safety assessment in rats and dogs displayed no adverse effects; however, toxicity concerns halted the progression and development of **5** into the clinic [67]. In an attempt to attenuate toxicity, modifications of the carboxamide moiety were investigated but did not produce fruitful results [68]. Furthermore, drug clearance remained high in rats.

GSK also probed the utility of the nitrogen atom of the piperidine ring and the length of the carbon linker attaching the piperidine ring to the tropane moiety in the 4,4-disubstituted scaffold [69]. A cyclohexylamine scaffold emerged from this study with compound **6** as the most potent derivative (HOS, $IC_{50} = 58$ nM). Structure–activity relationship (SAR) studies suggested that the preferred stereochemistry for the cyclohexyl ring was *cis* and that a 2-carbon linker was the optimal length.

Two novel series were investigated by GSK – DAB (2-phenyl-1,4-diaminobutane) and MDAB (2-methyl-2-phenyl-1,4-diaminobutane), which differ by a methyl group at the 2-position [70]. The objective was to determine the influence of the tropane moiety on the antiviral properties of the scaffold, and it was concluded that the MDAB series attached to an endo-tropane motif led to higher potency in PBL cells, while the same motif for the DAB series did not. The study revealed two potent compounds (**7**) from the DAB series that showed excellent anti-HIV potency in both HOS and PBL cells ($IC_{50} = 8$ and 60 nM, respectively) as well as moderate drug clearance in rats, but poor bioavailability.

In 2010, Zhang and coworkers reported a new scaffold which was structurally related to MVC [71]. The strategy behind their SAR was to identify a bioisostere for MVC's amide group that could be easily manipulated into other functional groups; thus, a carboxamide moiety was selected. Eleven compounds were synthesized in the study with the most potent compound **8** (Figure 2) with an $IC_{50} = 14.4$ nM in the RANTES-binding assay. Another new core scaffold based on the selection and assembly of privileged fragments from known CCR5 antagonists, particularly MVC, was reported. The scaffold features a novel propane-1,3-diamino bridge illuminated by compound **9** (Figure 2) that was found to have excellent activity against CCR5 in a RANTES-binding assay with an $IC_{50} = 0.253$ nM [72].

4.1.2. Monocyclic piperidine amides—TAK-220 (**10**, Figure 3) was developed from a lead compound discovered through a high-throughput screening at Takeda (Osaka, Japan). The piperidine-4-carboxamide moiety of TAK-220 (**10**) was found to be important for HIV activity and conferred metabolic stability that led to the clinical development of **10** [73]. TAK-220 was licensed to Tobira Therapeutics (San Francisco, CA) and reached phase I clinical trials as of 2008, but no further progress has been reported [74].

Inspired by Merck's (Kenilworth, NJ) 1,3,4-trisubstituted pyrrolidines, a series of 1,3,3,4-tetrasubstituted pyrrolidine compounds were reported in 2007 to improve the poor oral bioavailability of Merck's compounds [75]. This was accomplished by incorporating a hydroxyl group at the 3-position on the pyrrolidine ring to furnish nifedipine (**11**, TD-0232, Figure 3). Nifedipine displayed an $IC_{50} = 2.9$ nM in the RANTES-binding assay and possessed a favorable pharmacokinetic profile in rats and dogs. The nitro moiety of **11** was considered a possible mutagenic liability, and subsequent optimization (TD-0680, **24**) will be discussed later [76].

Pryde and coworkers were inspired by both the amide functionality of MVC and the piperidine amide moiety in Schering C (**12**, SCH-C, Figure 3) which led to SAR exploration [77]. Thirty compounds were synthesized in the first round of SAR with the lead compound displaying a modest IC_{50} of 145 nM in a gp160 cell–cell fusion assay. A second round of synthesis based around their lead resulted in two additional hits: compound **13** and **14** (Figure 3) with an IC_{50} of 1 and 3 nM, respectively. The authors note that compounds **13** and **14** are moderately lipophilic and may be a source of poor metabolic stability in liver microsomes. However, high permeability and weak hERG activity identify these compounds as promising starting points for preclinical development.

Barber and coworkers designed the 1-amido-1-phenyl-3-piperdinybutane series in search for an MVC-like compound without the tropane core to improve oral absorption [78]. The authors shifted toward a scaffold containing 4-heterocyclic substituted piperidines as bioisosteres of the amido group for improved permeability. Their initial lead compound **15** (Figure 3) (fusion assay, IC_{50} of 2.0 nM) contained a 1,3,4-triazole moiety that afforded higher potency and stability. Further optimization of the 4-heterocyclic-substituted piperidine series led to 1,2,4-triazole substitution with lead compound **16** (fusion assay, IC_{50} of 3.08 nM) that exhibited high oral bioavailability in rats, but did not extend the drug's half-life [79].

In 2011, compound **17** (Figure 3), a pyridine-2-yloxymethylpiperidin-1-ylbutyl amide, was discovered by Skerlj and coworkers [80]. Their strategy toward this new scaffold was to modify SCH-C (**12**) by opening the lower piperidine ring and finding a substitute for the oxime moiety. Compound **17** was their lead compound, which had strong inhibition against R5 HIV-1 replication in PBMCs (IC_{50} = 9 nM), but oral bioavailability in dogs was extremely poor (F = 6%). The group decided to move away from this series and to focus on modifications around the aniline to create a novel pyridine-2-ylmethylaminopiperidin-1-ylbutyl amide series [81]. Compound **18** (Figure 3) emerged as the lead compound with an IC_{50} = 0.2 nM in CCR5 fusion and 2.2 nM in PBMC assay. Compound **18** achieved a favorable *in vitro* and *in vivo* profile but had unfavorable hERG activity (IC_{50} = 0.25 μ M) [81]. Consequently, the series was abandoned due to cardiovascular concerns, and future works to mitigate hERG liabilities are ongoing.

A Y-shaped pharmacophore model that interacted with amino acids essential for CCR5 antagonism was used as the basis for a novel piperidine series. A patent disclosed in 2009 motivated additional SAR around the 'Y'-shaped model that led to the design of novel piperidine-4-carboxamide derivatives [83]. Piperidine **19** (Figure 3) was subsequently discovered and displayed excellent inhibition against CCR5 (calcium mobilization assay, IC_{50} = 25.73 nM) and anti-HIV activity (IC_{50} = 73.01 nM). Further pharmacokinetic properties were examined in a rat models that demonstrated 15% oral bioavailability. Compound **19** also exhibited modest inhibition of the hERG channel (15% at 3 μ M).

A novel piperidine scaffold [84] previously reported by Novartis (Basel, Switzerland) was found to have adverse cardiovascular side effects at submicromolar concentrations. In an attempt to dial out these undesirable properties, modifications to the diphenyl moiety were conducted which led to compound **20** (NIBR-1282, Figure 3) [85]. Compound **20** had an

IC₅₀ value of 5.1 nM in the CCR5-binding assay with no cardiovascular complications in animal models.

The diazaspiro[5.5]undeca-2-one compounds were derived from a 1-oxa-3,9-diazaspiro scaffold previously investigated by Roche [86,87]. A total of 27 derivatives were synthesized, and diazaspiro **21** (Figure 3) was identified as the most potent antagonist with an IC₅₀ = 30 nM in the RANTES-binding assay. Compound **21** had an attractive pharmacokinetic profile with bioavailability at 66% and a half-life of 4.19 h (PO [oral administration]) in rats and displayed no significant CYP or hERG inhibition.

4.1.3. Cyclic and acyclic urea-piperidines—Ernst and coworkers designed a novel imidazopiperidinetropane scaffold by merging the attractive features of MVC with other known CCR5 antagonists. The western portion of the molecule was converted from a cyclohexylamide to a urea moiety that greatly improved anti-HIV activity. Considerable SAR led to the identification of urea **22** (Figure 4) which potently inhibited MIP-1 β binding at IC₅₀ = 1 nM with 52% bioavailability and a terminal life of 10.7 h [88].

A high-throughput screening of the internal library at Berlex Biosciences (San Pablo, CA) uncovered a guanylhydrazone derivative (**37**, Figure 6) as an initial starting point for a novel series of urea derivatives [89]. In a second report, continued SAR around scaffold **37** (Figure 6) revealed that a 4-hydroxy piperidine analog had an improved level of potency against MIP-1 α binding to human CCR5/CD4-transfected HEK-293 (Human Embryonic Kidney-293) cells (IC₅₀ = 49 nM) [90]. The addition of a urea group at the 4-position on the piperidine led to the acyclic urea derivative **23** (Figure 4) as their most potent lead. Unfortunately, poor pharmacokinetic properties led to the abandonment of this series.

Building around the SAR that was successfully parlayed for Nifedipine (**11**, TD-0232), significant tuning of the *N*-substituents led to a urea-based derivative containing a methyl sulfonamide moiety in place of the nitro group as a potent CCR5 antagonist. Further modifications included the incorporation of an exotropane and meta-fluoro moiety on the phenyl ring that led to **24** (TD-0680, Figure 4) [76]. TD-0680 (**24**) showed excellent antiviral activity against eight different HIV-1 isolates in the PBMC assay and significantly inhibited replication at sub-nanomolar concentrations in cell–cell fusion assays mediated by R5-tropic envelopes of three major subtypes.

In 2010, GSK scientists revisited their 4,4-disubstituted scaffold to modulate the amine moiety, and a urea-based group was found to be the most active with promising pharmacokinetic properties in rats [91]. Additional SAR was elaborated in a subsequent publication at the acyl position to improve pharmacokinetics [92]. Compound **25** (Figure 4) emerged as a preclinical candidate with excellent antiviral activity (HOS, IC₅₀ = 8 nM) and 53% bioavailability in rats. Compound **25** advanced to PK (pharmacokinetic) studies in dogs and monkeys resulting in high bioavailability with low clearance in dogs, but low bioavailability (14%) and high clearance in monkeys.

Once GSK 163929 (**5**) was found to harbor toxicity, a significant redesign of the tropane benzimidazole was initiated and led to a *p*-cyanobenzyl urea derivative. Additional work

with scientists at Ono Pharmaceuticals (Osaka, Japan) resulted in the discovery of a novel pyridyl carboxamide series [67]. The effort began after a patent from ONO disclosed a CCR5 compound containing a urea moiety that was structurally similar to the urea moiety of **25**. The compounds were designed to preserve the urea scaffold while modifying the carboxamide region to afford a new pyridyl carboxamide series. Two compounds **26** and **27** (Figure 4) emerged as preclinical leads with an improved PK profile in rats.

A novel urea series reported in 2011 was found to have potent anti-HIV activity in HOS and PBL cell assays [93]. The original acyclic urea series was found to be unstable in fasted simulated intestinal fluid due to the acidic urea-nitrogen proton. Therefore, a cyclic urea series was developed to improve stability. Compound **28** (Figure 4) resulted from these efforts and demonstrated favorable potency (HOS and PBL, IC_{50} = 4 and 1.0 nM, respectively) and exhibited good *in vivo* PK properties in both dogs and monkeys (%*F* = 2.1 and 20.2 mL/min/kg, respectively).

Lastly, scientists at Genzyme (Cambridge, MA) applied a combination of mutagenesis, molecular modeling, and medicinal chemistry to uncover two structurally diverse acyclic and cyclic urea derivatives. These compounds, shown as **29** and **30** (Figure 4), had nanomolar IC_{50} activity with no cytotoxicity or hERG activity [94].

4.2. VVC- and AVC-inspired piperazines and diketopiperazines

In 2008, a novel scaffold was created from the 4-(piperazin-1-yl)quinolone moiety of a lead compound discovered via virtual screening and the amide moiety of MVC [95]. Twelve derivatives were synthesized around this scaffold. Of these 12 compounds, **31** (Figure 5) yielded an IC_{50} of 0.233 μ M, but this result was roughly 90-fold less potent than MVC. Despite this loss in potency, biological data obtained from strategic SAR modifications correlated with *in silico* experiments, which could provide insight into the future development of this scaffold.

In 2012, Dong and coworkers synthesized **32** (Figure 5), which was inspired by the structure of TAK-220 (**10**) and an octahydropyrrole[3,4-c]pyrrole moiety published in a patent by Roche [96]. Their objective was to replace the 4-benzylpiperidine moiety with an isosteric piperazine. Eighteen derivatives were synthesized using TAK-220 as a template; however, **32** emerged as the lead compound and demonstrated potent anti-HIV-1 activity (IC_{50} = 6.17 nM), low cytotoxicity (CC_{50} > 100), and high oral bioavailability (%*F* = 56), but a short half-life (0.76 h) [97].

In 2013, Liu and coworkers devised a Y-shaped pharmacophore model previously used by Hu in the synthesis of piperidine **19**. The Y-shaped model was used to design a novel piperazine series and was devised from previous work that elucidated the binding mode of both MVC and TAK-220 (**10**) [82]. The most potent lead was compound **33** (Figure 5) with an IC_{50} of 6.29 μ M in a CCR5-mediated fusion assay and an IC_{50} of 0.44 μ M in an anti-HIV assay.

From the same group, a 2015 report examined a fragment assembly-based strategy to develop a 2-methylpiperazin scaffold derived from the carboxamide moiety of TAK-220

(**10**) and the piperazine moiety from **33** [98]. The series resulted in compounds **34** and **35** (Figure 5) with nanomolar activity in CCR5 calcium mobilization assay (IC_{50} = 88 and 3.0 nM, respectively) as well as moderate antiviral activity (IC_{50} = 31.4 and 75.1 nM, respectively). In addition, no cytotoxicity was observed up to 10 μ M and thus provided an excellent starting point for further optimization.

INCB9471 (**36**), developed by Incyte (Wilmington, DE), was inspired by Schering-Plough's CCR5 antagonist VVC (**2**) [99]. Considerable SAR was conducted around the trifluorobenzyl moiety, which was known to interact strongly with the receptor. An indane was chosen after conformational analysis revealed it to be a suitable replacement that provided enough rigidity while maintaining the correct orientation. INCB9471 is comparable in potency to SCH-D and inhibited R5 HIV-1 strains at IC_{50} values of 0.36 and 0.16 nM (ADA and Ba-L, respectively). INCB9471 exhibited excellent *in vivo* properties characterized by a drug half-life, low systemic clearance, and high volume of distribution in rats and dogs. Oral bioavailability was at maximum in rats and near maximum in dogs (% *F* = 100 and 95). INCB9471 advanced to phase I and II clinical trials and was found to be safe, but development was halted for other business priorities.

4.3. Miscellaneous scaffolds and approaches

In 2007, Berlex Biosciences conducted a high-throughput screening of their internal library through a 125 I-MIP-1 α -binding assay on human CCR5/CD4 and found compound **37** (Figure 6) to have an IC_{50} = 2.2 μ M in Ca^{2+} flux assay [89]. The study described the synthesis of 47 derivatives where the moiety at the R-position was replaced with a benzyl group, aryl group, or guanylhyazone. It was discovered through the course of their study that the guanylhyazone moiety of **37** was not required for potency, but rather a tertiary amine such as **38** (IC_{50} = 0.6 μ M) was well tolerated. Compound **38** was selected for further exploration of the PK profile [90].

A new hexahydropyrrol[3,4-c]pyrrole moiety was identified through a high-throughput screening from a library of compounds at Roche [100]. The hexahydropyrrol[3,4-c]pyrrole moiety was considered to serve as a bioisostere for the tropane unit of MVC. The lead compound demonstrated excellent antiviral activity (IC_{50} = 7 nM) but was plagued by metabolic instability. In a subsequent report, detailed SAR around the *N*-substituted pyrazole subunit was conducted [101]. Efforts to optimize antiviral activity and metabolism led to compound **39** (Figure 6, antiviral IC_{50} = 11 nM). The authors remarked on the inability to improve *in vitro* metabolic stability while maintaining good antiviral activity, which ultimately led to the abandonment of this series. In addition, two parallel series were examined where significant SAR around the secondary amide was probed to improve membrane permeability. Compounds **40** and **41** (Figure 6, antiviral IC_{50} = 1.2 and 17 nM, respectively) were identified as lead targets [102,103].

Compound **42** (Figure 6) was discovered during an investigation of two novel series called DAB (2-phenyl-1,4-diaminobutane) and MDAB (2-methyl-2-phenyl-1,4-diaminobutane) by scientists at GSK [70]. The study revealed compound **42** from the DAB series as containing excellent potency in both HOS and PBL cells (IC_{50} = 8 and 1 nM, respectively) as well as moderate drug clearance in rats, but poor bioavailability.

Lemoine and coworkers broadened the scope of the hexahydropyrrol[3,4-c]pyrrole motif and synthesized an endo 5-amino-3-azabicyclo[3.3.0]octane moiety. The 5-amino-3-azabicyclo[3.3.0]octane was explored as a replacement both for the tropane subunit of MVC and for the 4-aminopiperidine subunit in both VVC (**2**) and INCB9471 (**36**) to furnish **43** and substructure **44** (Figure 6) [104,105]. Each derivative was examined in a [¹²⁸I]-RANTES-binding assay and an R5 antiviral assay, and the [3,4-c]pyrrole motif was found to be a suitable replacement in MVC, VVC, and INCB9471.

Many companies and research groups have broadened their search for novel scaffolds to target CCR5. Yang and coworkers in 2006 screened secondary metabolites from the microbial extracts of fungi, actinomycetes, or bacteria [106]. A high-throughput screening utilizing a radioactive CCR5-binding assay that inhibited RANTES binding was conducted on the extracts and led to the discovery of two tetramic acids Sch210971 (**45**, Figure 6) and Sch210972 (**46**, Figure 6) with an IC₅₀ of 1.2 μM and 79 nM, respectively. The following year, the same group identified a third metabolite Sch213766 (**47**, Figure 6) that had an IC₅₀ of 8.6 μM [107]. Tetramic acid compounds are known antimicrobials, but it is the first time that it has been described to have CCR5 activity.

Drug compliance continues to be a challenge for many patients bound to lifelong antiviral therapy. A drug candidate that demonstrates low clearance and a relatively long biological half-life has the potential to reduce dosing frequency and improve compliance. One in particular is to attach a small-molecule drug to an antibody creating chemically programmed antibodies (cpAbs). To produce a cpAbs, one can utilize the lysine residue of murine catalytic monoclonal antibody 38C2, which is capable of aldolase activity, and selectively label it with *N*-acyl-β-lactams. Another process is to attach a polyethylene glycol (PEG) linker to the small molecule in a process called PEGylation to improve properties. Asano explored both methods using MVC as the small molecule to improve properties [108]. The cpAbs **48** and PEGylated **49** (Figure 6) both had comparable IC₅₀ values in the neutralization assay at 7.7 and 5.6 nM, respectively. Most importantly, both compounds were stable and held activity for up to 10 days compared to the parent drug MVC in human serum. Toxicology studies of the conjugates are unknown and thus warranted further study.

5. Dual chemokine CCR5 antagonists

5.1. Dual CCR5/CCR2 antagonists

The finding that MVC had a dyslipidemic effect in HIV patients spurred the interest that the multiple chemokines that bind to CCR5 may play a role in regulating the immune system in ways that should be extremely beneficial to HIV-infected patients. The promiscuous chemokine ligands that bind CCR5 (CCL 3, 4, 5, and 8) have commonality with other chemokine receptors including CCR1 (CCL 3, 4, and 5), CCR2 (CCL8), CCR3 (CCL5), CCR4 (CCL3 and 5), and CCR8 (CCL4) [109]. This redundancy among chemokines and their receptors also increases the likelihood of finding CCR5-based small molecules that bind to the other CCR receptors in this list. The first such reported multiple chemokine approach in the HIV setting has been the serendipitous finding of dual CCR5–CCR2 activity.

TAK-779 (**50**, Figure 7) was the first non-peptide CCR5 antagonist reported in 1999 [110]. A high-throughput screening of Takeda's chemical library using both [¹²⁵I]-RANTES and CHO/CCR5 assay identified this quaternary ammonium salt as one of their leads and a new CCR5 antagonist [111]. Optimization of TAK-779 structure generated a diverse series of benzoxapines, dihydronaphthalenes, and benzocycloheptene derivatives. From these derivatives, the benzocycloheptene scaffold in **50** was the most selective toward the CCR5 receptor with nanomolar affinity but displayed activity toward CCR2 (IC₅₀ = 27 μM) as well. The *in vitro* anti-HIV replication assays showed that **50** had strong potency with IC₅₀ values against MAGI cells and PBMCs at 1.2 and 3.7 nM, respectively. Although TAK-779 became the lead compound, intrinsic issues with oral absorption stemming from the quaternary ammonium moiety required additional optimization to the core structure. TAK-652 (Cenicriviroc, **51**, Figure 7) arose after optimizing the quaternary ammonium moiety to a sulfoxide functional group and a ring expansion of the [6,7]-fused 1-benzazepine to a [6,8]-fused -1-benzazocine [112]. A 10-fold increase in potency in the anti-HIV replication assay was observed for TAK-652 against six R5-clinical strains in PBMCs with a mean IC₅₀ value of 0.25 nM. Inhibition of both receptors CCR2 and CCR5 was found to be similar with TAK-652 at 5.9 and 3.1 nM, respectively, and good oral absorption in rats, dogs, and monkeys was also observed. With high potency and improved oral bioavailability relative to TAK-779, TAK-652 was selected for further study and is currently in phase IIb clinical trials for treatment of HIV infection. This compound, now known as Cenicriviroc, is the most advanced CCR5 antagonist currently in clinical trials. Although the observation that MVC reduces dyslipidemia in HIV patients undergoing HAART treatment, the additional clinical benefits of blocking CCR2 with TAK-652 versus CCR5 alone are unknown at this time as CCR2 is not involved in HIV infection.

Novartis disclosed a series of indole derivatives that are dual CCR2/CCR5 inhibitors in a patent application [113]. Their initial hit compound was found via a high-throughput assay against CCR2 antagonists of Novartis' chemical library [114]. Lead compound NIBR-6465 (**52**, Figure 7) exhibited high potency in both human and rat CCR2/CCR5 receptor assays [115]. In 2010, clinical trials with NIBR-6465 for HIV were disclosed, and **52** has successfully completed phase I studies [114,116].

Other dual CCR2/CCR5 inhibitors have been reported, but many have been pursued as anti-inflammatory agents. Several small molecules have been evaluated to target both receptors simultaneously, but evaluation of HIV activity was not performed, and no HIV data were provided. A high-throughput screening of the SmithKline Beecham Library identified an indole compound that was later optimized to **53** (Figure 7) with K_i values of 50 and 4.3 nM against CCR2 and CCR5, respectively [117]. Merck also investigated **54** (Figure 7) to combat various autoimmune diseases such as rheumatoid arthritis and multiple sclerosis [118]. The IC₅₀ of **54** toward CCR2 was 4 nM and also inhibited CCR5 at 10 nM. Lastly, a collaboration between Incyte Corporation and Pfizer led to the discovery of dual CCR2 and CCR5 antagonist INCB10820/PF-4178903 (**55**, Figure 7) as a therapeutic agent to curb the recruitment of monocytes to sites of inflammation [119]. The IC₅₀ for binding of **55** to CCR2 and CCR5 was 3.0 and 5.3 nM, respectively.

5.2. Dual CCR5/CXCR4 receptor compounds and approaches

Targeting both CCR5 and CXCR4 has potential to simultaneously thwart both X4- and R5-tropic viruses and would obviate the need for a tropism test; however, this concept has garnered sparse attention. Since tropism shifts occur in both treatment-experienced and treatment-naïve HIV-infected patients, targeting both R5- and X4-tropic forms of the virus would provide a drug of more broad utility. Initial studies have demonstrated that this concept could work in practice. A substantial decrease (93%) in viral replication when an excess of CCL5 (ligand for CCR5) and CXCL12 (ligand for CXCR4) is present in cell cultures infected with dual-tropic HIV [120]. Furthermore, it was also shown that a combination of small-molecule antagonists (MVC and AMD3100) also abrogated replication of the same dual-tropic HIV strain. This work validated the idea that combination CCR5/CXCR4 therapy has the capacity to block HIV entry and prevent replication and could target both viruses simultaneously.

The search for a dual CCR5/CXCR4 HIV entry inhibitor has been reduced to practice, and the *N*-pyridinylmethyl cyclam AMD3451 (**56**, Figure 7) was reported as the first dual-tropic inhibitor in 2004 [121]. It is interesting to note that AMD3451 is structurally similar to AMD3100, which binds CXCR4 but not CCR5. AMD3451 was moderately active against X4 HIV-1 strains such as IIIB and NL4.3 with an IC₅₀ of 1.2 and 1.8 μM, respectively, and also against the R5-tropic strain Ba-L with an IC₅₀ value of 23.9 μM. Furthermore, dual-tropic HIV strains were inhibited by AMD3451 with an IC₅₀ of 26.5 μM. Although AMD3451 does not have suitable potency for clinical use, its discovery serves as a proof-of-concept toward the future development of novel dual-tropic CCR5/CXCR4 antagonists.

A pyrazolo-piperidine series with CCR5/CXCR4 activity were recently discovered at Emory University through a combination of virtual high-throughput screening and Bayesian statistical partitioning [122]. The screening revealed a substructure with weak HIV inhibition (25 μM, MAGI assay) that consisted of a structural pyrazolo-piperidine core. A secondary screen led to compound **57**, which demonstrated low micromolar HIV activity (MAGI assay). Subsequently, a cell–cell fusion assay established that **57** inhibited fusion similar to TAK-779 against R5-tropic HIV. However, **57** did not displace radioligand [¹²⁵I]-CXCL12 from CXCR4. Interestingly, **57** exhibited activity in Ca²⁺ flux GPCR signaling which raises the possibility of negative allosteric modulation of CXCR4. Furthermore, **57** also displayed non-nucleoside reverse-transcriptase inhibitory activity both in time-of-addition studies and in an isolated HIV-1 reverse-transcriptase enzyme assay (IC₅₀ = 9.0 μM). Additional studies with **57** revealed that its NNRTI activity is the primary mechanism of action; however, current efforts are in motion to enhance dual CCR5/CXCR4 inhibition while dialing out NNRTI activity.

6. Potential challenges for CCR5 antagonists: resistance mechanisms

Clinical experience has repeatedly demonstrated that HIV is prone to drug resistance. Studies with VVC [123–126], MVC [127,128], AVC [129], and AD101 [130] have concluded that a shift in co-receptor usage (CCR5 → CXCR4) is not the operative mechanism behind resistance to CCR5 antagonists *in vitro* or *in vivo*. Rather, adaptive changes in the gp120 V3 loop allows HIV to tighten its grip on CCR5 to use the drug-bound

form of the receptor [124–126,131–133]. In several cases, inhibition is restored with monoclonal antibodies, RANTES, or other bulky CCR5 ligands that completely blockade HIV from approaching the active site of the receptor. In 2012, a novel mechanism of resistance was reported with MVC [134]. In that study, resistance to MVC was dependent on a single mutation in the C4 region of gp120 (N425K), which is known to interact with F43 within the D1 domain of CD4, not CCR5. Subsequent docking and modeling studies reveal that the K425 mutant generates new cation- π interactions with F43 on CD4 and suggest that enhanced interactions with CD4 circumvents the need for CCR5 binding which may be the source of MVC resistance. Sequence changes in the fusion peptide gp41 have also been reported to confer resistance to VVC [135,136], and other strain-specific V3 loop mutations contribute to MVC resistance *in vivo* [137]. Recently, the more advanced dual CCR5/CCR2 antagonist TAK-652 was the subject of resistance studies (R5 HIV-1 isolate KK) showing an escape virus with 200,000-fold reduction in activity [138]. Furthermore, the TAK-652-resistant virus was incapable of using CXCR4 as a co-receptor, confirming that no tropism shift had occurred during viral evolution. Taken together, it is clear that CCR5 antagonists are not immune to selective forces and are susceptible to unprecedented mechanisms of resistance, which do not include tropism shifts to X4 but rather HIV using drug-occupied CCR5 receptor.

A single report describing one strategy to overcome this resistance mechanism shows potential. The CCR5 antagonist TD-0680 (25) was reported to have retained efficacy against a dual TAK-779/MVC-resistant variant of HIV [139]. The authors show that TD-0680 had increased efficacy against HIV-1_{JR-FL} and HIV-1_{Ba-L} viruses in cell fusion and PBMC assays versus MVC and TAK-779. Furthermore, they show that TD-0680 retained activity against the dual TAK-779/MVC-resistant strain HIV-1_{Ba-L5.6r}. They proposed that TD-0680 had increased interactions with the ECL2 (extra-cellular loop-2) region of CCR5, resulting in a more effective disruption of gp120 binding than other CCR5 antagonists. However, the failure of a CCR5 antagonist in the clinic from resistance development in patients during drug administration remains to be seen.

7. Expert opinion

The success of MVC in patients has set a high standard for next-generation CCR5 entry inhibitors as both VVC and AVC failed in the clinic mainly due to the lack of efficacy. However, the inadequacies of hepatotoxicity and use in only treatment-experienced patients place CCR5 antagonists in a secondary role in the treatment of HIV. The ongoing experimental and clinical research on CCR5 antagonists for HIV treatment has resulted in several significant second-generation agents with novel structural features and dual chemokine activity. These follow-up leads based on MVC and the other second-generation CCR5 compounds have failed to produce a clinical candidate or failed to reach advanced-stage clinical trials (Table 1, PF-232897, TAK-220, and INCB9471) with MVC itself remaining superior. Surprisingly, the only CCR5 antagonist currently in advanced clinical trials for the treatment of chronic HIV infection is Cenicriviroc (TAK-652, **51**). In addition to CCR5, Cenicriviroc also binds to CCR2 and may confer immunologic, cardiovascular, and metabolic benefits in HIV-infected individuals who experience low levels of immune activation during prolonged HAART treatment. MVC has been observed to provide

metabolic benefits, but this effect is mediated solely through the CCR5 receptor, as no intrinsic CCR2 activity has been reported with this drug. The additional physiologic benefit these drugs may bring is unlike any other medication in HAART as they are the only type of therapy to interact with a mammalian target and as such could be a game changer elevating the status of these drugs. However, it is unknown if the dual CCR5/CCR2 activity of Cenicriviroc will be significantly different or superior to MVC at this time. Unfortunately, MVC and Cenicriviroc still require the TROFILE test to prequalify patients that harbor only R5 virus. The development of a dual CCR5/CXCR4 HIV entry agent or coadministration of MVC with another suitable CXCR4 antagonist will circumvent the need for the TROFILE test in the future and could elevate such drugs and combinations into a first-line treatment option in drug-naïve patients. Dual chemokine-based agents (CCR5/CXCR4 and/ or CCR5/CCR2) are the entry inhibitors of tomorrow and have the potential to provide benefits in comparison to MVC such as curbing inflammation associated with chronic HIV infection in treatment-experienced patients with undetectable viral loads and eliminating the need for tropism testing. Furthermore, due to the chemokine ligand redundancies, it is likely that other CCR receptors could be added to this approach.

In closing, the potential advantages of reduced resistance profile and synergism with other HIV agents have yet to be realized. Furthermore, the prophylactic potential of MVC and other CCR5 antagonists against HIV remains to be seen in human trials. Lastly, there are other factors that are significant for the future development of CCR5 antagonists but lie outside the context of this review. One is the development of the CCR5 antibody PRO140, which requires infrequent dosing (mono or biweekly subcutaneous injections) due to the long half-life (3.4 days) in humans [140]. The successful development of this protein for use in the HIV setting may stimulate further interest in approaches such as the antibody/macromolecule/MVC conjugates (48 and 49) presented in this review or other technology (e.g. extended-release formulations) that greatly increase drug half-lives. The other significant findings are the use of MVC in prevention of host-versus-graft disease in stem-cell transplant patients increasing the clinical use of this agent outside of HIV [141] and trials of Cenicriviroc in patients with liver disease (non-alcoholic steatohepatitis and primary sclerosing cholangitis [PCS]) to reduce fibrosis [142]. The favorable outcome of these efforts may also resurrect the therapeutic and commercial interest in research and development activities to find newer and more efficacious CCR5 antagonists with expanded utility.

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Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

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Article highlights

- A brief background is provided on CCR5 receptor biology and pharmacology, the dual-tropism behavior of HIV (R5/X4), and the significance of the δ -32 deletion leading into the introduction of CCR5 entry inhibitors.
- A short review on the clinical outcomes of the first generation CCR5 antagonists is presented with a focus on the only FDA approved drug in this class - Maraviroc.
- A thorough review of the drug discovery and research efforts on second-generation CCR5 antagonists is provided identifying various drug scaffolds, preclinical and clinical candidates including PF-232798 and INCB9471.
- The discovery of dual-chemokine (CCR5 and CCR2 or CXCR4) antagonists is discussed culminating with the identification of the dual CCR5/CCR2 antagonist TAK-652/Cenicriviroc as the most advanced candidate currently in Phase II trials.
- A final examination of the upside potential, challenges and future for CCR5 antagonist development for HIV is provided. This includes the expanded role of Maraviroc in curbing immunologic and metabolic disorders, the challenges with the tropism test, the emergence of dual chemokine receptor pharmacology, drug-bound receptor resistance development, and use in non-HIV applications.

This box summarizes key points contained in the article.

Figure 1.
First generation CCR5 antagonists.

Figure 2.
Tropane-based CCR5 antagonists.

Figure 3.
Piperidine-amide CCR5 antagonists.

Figure 4.
Piperidine-urea CCR5 antagonists.

Figure 5.
Piperazine CCR5 antagonists.

Figure 6.
Other classes of CCR5 antagonists.

Figure 7.
Dual CCR5/CCR2 and CCR5/CXCR4 compounds.

Table 1

Summary of clinical studies on CCR5 antagonists.

Drug name/ sponsor	Clinical stages and trials	Outcomes and significant findings
Maraviroc (MVC) Pfizer/ViiV Healthcare	Approved by the Food and Drug Administration in 2007 for treatment-experienced patients with only CCR5 using virus (MOTIVATE 1 and 2). Treatment-naïve patients in comparison to tenofovir/FTC. MVC versus efavirenz in combination with zidovudine-lamivudine in treatment-naïve patients (MERIT). PrEP therapy in MSM. Microbicide Trials Network: phase I, MVC, dapivirine, and dapivirine-MVC combination vaginal ring Phase I/II. Allogeneic bone marrow transplantation for leukemia.	Lower-dose favored (150 mg) as hepatotoxicity occurs with the 300 mg dose. TROFILE tropism testing pre-requirement. MVC had inferior performance: smaller reduction in viral load. Faster recovery of CD4+ T cells. Reduced metabolic abnormalities and dyslipidemia. Some lack of efficacy in MVC arm. Recruiting. MVC ring did not meet drug concentration end points. Reduction in graft rejection in host-vs.-graft disease.
Vicriviroc (VVC) Schering	Phase II/III in treatment-experienced patients. Halted/abandoned (VICTOR E1, 3, and 4)	Liver malignancies. Viral rebound. Viral reduction end points not met in phase III trials.
Aplaviroc (APL) GlaxoSmithKline	Phase I/II in treatment-experienced patients. Halted/abandoned.	Severe hepatotoxicity. Poor/under effective efficacy.
INCB9471 Incyte	Phase I/II. Halted.	Discontinued for business reasons – portfolio switch. Seeking development partner to continue.
PF-232798 Pfizer/ViiV Healthcare	Phase I in healthy volunteers 250 mg well tolerated. Phase II discontinued.	Drug was discontinued due to the success of MVC.
TAK-220 Tobira/Takeda	Reported in phase I. No conformation or data available.	Likely drug was put on hold and development shifted to TAK-652.
Cenicriviroc (CVC, TAK-652) Tobira/Takeda	Phase I – completed. Phase IIB. Ongoing, includes with FTC/TDF with Sustiva/Truvada with dolutegravir, midazolam HIV-neurocognitive impairment Phase IIB – HIV-infected and HIV-non-infected patients: For nonalcoholic steatohepatitis in adults with liver fibrosis (CENTAUR) For primary sclerosing cholangitis (PERSEUS)	Drug was well tolerated, and no patient withdrawals were noted. Terminated. Completed. Results not reported. Completed. Results not reported. Recruiting patients. Ongoing. Enrolled non-HIV- and HIV-infected patients. Recruiting patients.

FTC: 5-fluoro-3'-thia-2',3'-dideoxycytidine; MSM: men having sex with men; VICTOR: Vicriviroc in Combination Treatment With Optimized ART Regimen; TDF: Tenofovir Disoproxil Fumarate; CENTAUR: Efficacy and Safety Study of Cenicriviroc for the Treatment of Nonalcoholic Steatohepatitis (NASH); PERSEUS: Preliminary Efficacy and Safety of Cenicriviroc in Adult Subjects With Primary Sclerosing Cholangitis.