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Selective and Dual Targeting of CCR2 and CCR5 Receptors: A Current Overview

Anna Junker, Artur Kamil Kokornaczyk, Ann Kathrin Strunz, and Bernhard Wünsch

Abstract The chemokine receptor 2 (CCR2) and chemokine receptor 5 (CCR5) are important mediators of leukocyte trafficking in inflammatory processes. The emerging evidence for a role of CCR2 and CCR5 receptors in human inflammatory diseases led to a growing interest in CCR2- and CCR5-selective antagonists. In this review, we focus on the recent development of selective CCR2/CCR5 receptor ligands and dual antagonists. Several compounds targeting CCR2, e.g., INCB8761 and MK0812, were developed as promising candidates for clinical trials, but failed to show clinical efficacy as presumed from preclinical models. The role of CCR5 receptors as the second co-receptor for the HIV-host cell fusion led to the development of various CCR5-selective ligands. Maraviroc is the first CCR5-targeting drug for the treatment of HIV-1 infections on the market. The role of CCR5 receptors in the progression of inflammatory processes fueled the use of CCR5 antagonists for the treatment of rheumatoid arthritis. Unfortunately, the use of maraviroc for the treatment of rheumatoid arthritis failed due to its inefficacy. Some of the ligands, e.g., TAK-779 and TAK-652, were also found to be dual antagonists of CCR2 and CCR5 receptors. The fact that CCR2 and CCR5 receptor antagonists contribute to the treatment of inflammatory diseases renders the development of dual antagonists as promising novel therapeutic strategy.

Keywords Atherosclerosis, AZD5672, AZD5672, CCR2, CCR5, Chemokine receptors, GSK163929, hERG, HIV-1, INCB10820, INCB3284, INCB3344, INCB8761, Inflammation JNJ17166864, Maraviroc, MCP-1, MIP-1, MK0483, MK0812, PF-232798, PF-4136309, PF-4254196, RANTES, RS504393, SKB3380732, TAK-220, TAK-652, TAK-779, UK-107,543, UK-347,503, UK-427,857

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Abbreviations

5-HT	5-Hydroxytryptamine
AT	Angiotensin receptors
CHO	Chinese hamster ovary
CYP450	Cytochrome P450 enzyme
EMEA	European Medicines Evaluation Agency
ET	Endothelin receptor
FDA	Food and Drug Administration
GPCR	G-protein-coupled receptor
hERG	Human ether-a-go-go-Related Gene
HIV	Human immunodeficiency virus
IC_{50}	Half maximal inhibitory concentration
MS	Multiple sclerosis
PET	Positron emission tomography
RA	Rheumatoid arthritis
SAR	Structure affinity relationship
SP	Spiropiperidine
TdP	Torsades de pointes
TM	Transmembrane domain

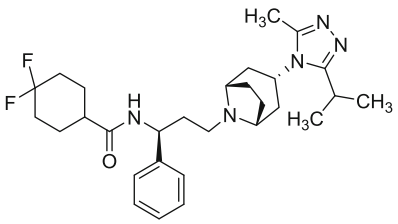


Fig. 1 **1** (UK-427,827, maraviroc, Celsentri®)

1 Introduction

In the last 25 years, chemokines and their receptors have become promising targets in many fields of research. Because the chemokine receptors 2 (CCR2) and 5 (CCR5) represent highly interesting candidates of the chemokine receptor family, much of investigations have recently been carried out in the development of ligands for these receptors. Maraviroc (**1**) is the first and to date the only FDA- and EMEA-approved drug on the market, targeting the CCR5 receptor (see Fig. 1) [1, 2]. The intention of this review is to elucidate the structure activity relationships of various small-molecule CCR2 and CCR5 ligands. The focus will be on the receptor binding affinity, antiviral activity for the treatment of HIV, and chemotactic activity for the treatment of atherosclerosis. In addition to the receptor binding affinity, many further aspects, which play a crucial role in drug development, will be discussed, e.g., physicochemical properties, lipophilicity, and the affinity to the human Ether-à-go-go-Related Gene (hERG), a K^+-channel which would lead to severe side effects induced by the compounds.

The CCR2 receptor has become a promising target in the therapy of atherosclerosis. The concerted action of the chemokine ligand CCL2 and the CCR2 receptor plays an important role in the recruitment of monocytes from the bone marrow into the arterial wall, which is known to be an early key step in atherosclerotic plaque formation. Lesions of the arterial endothelium are caused by mechanic injury or toxins and lead to migration of monocytes into the subendothelium that is mediated by adhesion molecules and chemokine receptors. In the artery wall, monocytes differentiate into macrophages, which develop to foam cells by taking up blood lipids [3–5]. Advanced plaques become unstable and can suddenly rupture. They expose their content to the blood, leading to platelet aggregation and occlusion of the blood vessel. Thrombosis, stroke, and myocardial infarction result as serious long-term complications. With regard to the increasing number of patients, the use of small-molecule CCR2 antagonists in atherosclerosis has attracted significant attention in the last years [6]. The CCR2 receptor is linked also to progression and development of other inflammatory diseases like multiple sclerosis (MS) and rheumatoid arthritis (RA) [7].

The CCR5 and the CXCR4 receptors are mainly known as co-receptors required for the development of the HIV-1 infection [8]. The binding of gp120 of the virus to CD4 receptors on T-lymphocytes and macrophages leads to a conformational

change of gp120 and enables the interaction with the CCR5 co-receptor. This triggers conformational changes in gp41, which leads to the fusion of the virus with the host cell [9]. At the beginning of an infection, the vast majority of the transmitted virus strains is M- or R5-tropic and uses the CCR5 receptor as a co-receptor. The T- or X4-tropic virus strains, which use the CXCR4 receptor as a co-receptor, are associated with an advanced disease progression [10]. A genetic polymorphism of the CCR5 receptor, characterized by a deletion of 32 bp in the gene segment encoding the receptor (CCR5Δ32 bp), results in a lack of function of the CCR5 receptor in homozygote individuals. These CCR5Δ32 bp mutations are found in 1–5% among uninfected Caucasian population and is exceedingly rare in infected patients (<0.1%), which indicates that CCR5Δ32 bp homozygotes are strongly resistant against HIV-1 infection [11–13]. The CCR5Δ32 bp mutant has also been linked to reduced susceptibility to coronary artery diseases and myocardial infarction [14, 15]. CCR5Δ32 bp polymorphism, as well as the function of the CCR5 receptor as a co-receptor for the HIV-host cell fusion, stimulated the beginning of several drug development programs by different pharmaceutical companies since the early 2000s.

In this review, we will focus on selective CCR2 (Sect. 2) and CCR5 (Sect. 3) receptor ligands, which can be used in the treatment of several immunological diseases including arthritis [16], asthma [17], multiple sclerosis [18], vascular diseases [19, 20], and HIV-1 infection [7, 21]. The fact that the antagonism of both CCR2 and CCR5 receptors may contribute to the treatment of inflammatory diseases makes the development of dual antagonists attractive. Dual CCR2 and CCR5 antagonists will be discussed in Sect. 4.

2 CCR2 Receptor Ligands

The CCR2 receptor plays an important role in an inflammatory response and is involved in several diseases of the immune system including atherosclerosis. The interaction of the chemokine CCL2 with the CCR2 receptor is responsible for the recruitment of blood monocytes to the site of inflammation and is also an early key step in the pathogenesis of atherosclerosis. The CCR2 receptor represents a promising therapeutic target for the treatment of atherosclerosis and is discussed as potential PET (positron emission tomography) target for diagnostic use [7]. Current atherosclerosis treatments are restricted to manipulation of indirect mechanisms, e.g., the modulation of cholesterol or triglyceride concentration, control of homoeostasis, or reduction of other risk factors associated with the metabolic syndrome. With regard to millions of patients (and numbers rising) who suffer under this chronic inflammation, CCR2 antagonists have attracted substantial attention during the past years [20].

Early developments of CCR2 antagonists in the late 1990s have been already reviewed elsewhere [6, 22–24]. This chapter will analyze structural features from different classes of CCR2 antagonists that were published until August 2013.

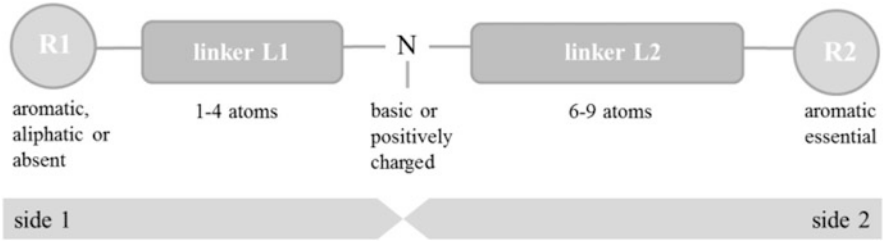


Fig. 2 General pharmacophore model for CCR2 antagonists, modified according to ref. [27]

We will compare them with regard to their structure activity relationships (SARs), explain strategies that led to increasing CCR2 affinity and selectivity, and elucidate the influence on the CYP system and hERG inhibition as well. The affinity of ligands to the CCR2 receptor is usually determined in the radioligand displacement assays, where the chemokine [^{125}I] CCL2 is used as the radioligand [25]. Chemotactic assays are generally used to determine the compounds' ability to inhibit the CCL2-stimulated chemotaxis in human peripheral blood monocytes [26].

2.1 Pharmacophore Model

Several series of CCR2 antagonists from different structural classes have been described in patents and publications. The majority of known CCR2 antagonists consist of a basic center flanked by two lipophilic residues as aromatic rings or one aromatic and one aliphatic moiety as demonstrated in the pharmacophore model in Fig. 2. The basic amine or quaternary ammonium ion of the ligand essentially anchors a small-molecule ligand to Glu291 (in the transmembrane domain 7 (TM7)) of the CCR2 receptor by a salt-bridge formation [28].

The aryl or heteroaryl motif R2 on side 2 is another essential feature of this pharmacophore model. The substitution pattern of this aromatic system greatly influences the CCR2 receptor affinity. The linker L2 of 6–9 atoms with lipophilic or polar, peptidic or saturated, and unsaturated or aromatic structural elements is well tolerated by the receptor and determines the specificity of binding.

The left part of the molecules tolerates more variations: the moiety R1 can be an aliphatic or aromatic ring. The linker L1 can be short and aliphatic (1–4 atoms), can be part of a ring system, or it can represent an aliphatic system in case of absence of a ring system [27]. Compounds with an aromatic ring in the R1 position show interactions with the hERG channel [29].

Most of the CCR2 ligands, discussed in this chapter, correspond to the described pharmacophore model. The report of the crystal structure of the CXCR4 receptor [30], which has a high sequence homology with the CCR2 receptor, initiated various structure-based approaches in the CCR2 ligand design and the investigations of the ligand-receptor interactions.

2.2 hERG Channel Interaction

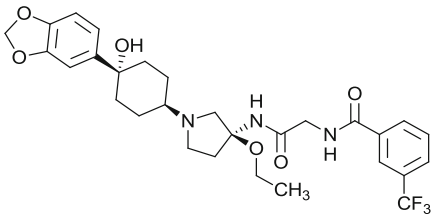
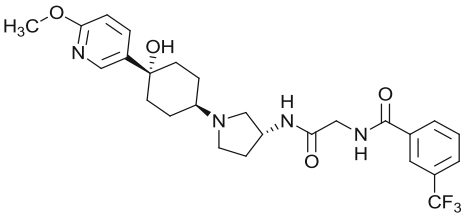
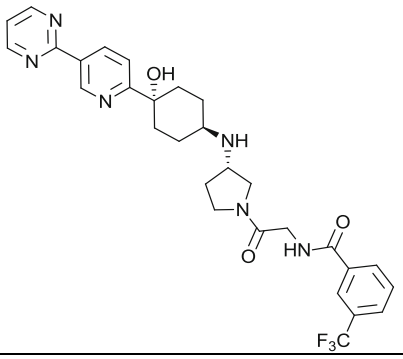
Predominantly, lipophilic amines that contain a central basic amine flanked by two lipophilic moieties according to the CCR2 pharmacophore model (Fig. 2) show high affinity for the human Ether-a-go-go-Related Gene (hERG) K^+-channel [29, 31]. The aromatic moieties of a drug form π–π-stacking and hydrophobic interactions with the residues Phe656 and Tyr652 from the large cavity of the hERG channel [32]. The hERG gene encodes the α-subunit of the inwardly rectifying K^+-channel, which is highly expressed in the human heart. This channel is involved in the repolarization of the cell. The mutation of the hERG gene or a channel blockade by drugs can lead to prolongation of the QT interval in the electrocardiogram in severe arrhythmia.

High sensitivity of the hERG channel to a blockage by many drugs and with the resulting cardiovascular adverse events like torsades de pointes (TdP), which can degenerate into ventricular fibrillation, led to the requirement of regulatory agencies that the effect of novel drugs on the hERG channel has to be investigated and reported [33]. Hence, considering hERG channel blockade is essential to improve cardiovascular safety of novel CCR2 antagonists. The increase of overall polarity of the drugs by introduction of hydrophilic ring systems and substituents on side 2 has been a successful strategy to eliminate hERG inhibition [31]. Further, successful approaches are the attenuation of the pK_a value of the basic amine, modification of its steric environment, and the formation of zwitterions [32, 34].

2.3 Pyrrolidine-Based CCR2 Ligands

Incyte's INCB3344 (**2**), one of the first potent pyrrolidine derivatives with a 3,4-methylenedioxyphenyl residue (Table 1), was well investigated in receptor binding and chemotaxis assays with human (hCCR2) and murine (mCCR2) receptors [35]. Despite the fact that human and murine CCR2 receptors show high sequence homology, binding affinities of ligands differ considerably among species [36]. INCB3344 (**2**) showed the IC_{50} values of 5.1 nM in hCCR2-binding and 3.8 nM in chemotaxis assay. **2** has been used as a tool in rodent in vivo efficacy models for multiple sclerosis, arthritis, and obesity and was effective in lowering macrophage levels in the targeted tissue [25, 26, 37]. Despite a high selectivity over other chemokine receptors, INCB3344 (**2**) was not a suitable clinical candidate due to its moderate hERG binding activity (IC_{50} = 13 μM) and inhibition of CYP 3A4 [6, 35]. This data led to further structural modifications of **2**. The removal of the ethoxy group at the quaternary carbon at position 3 of the pyrrolidine ring led to a loss of the mCCR2 affinity but retained the hCCR2 affinity. Previous SAR studies proved that the trifluoromethylphenyl residue on side 2 was crucial for the CCR2 binding affinity. The (*R*)-configuration at the position 3 of the pyrrolidine ring was also known to be important for the CCR2 affinity [38, 39]. The optimization

Table 1 CCR2 antagonists with pyrrolidine structure, inhibitory effects on CCL2 binding to human CCR2 receptor

Compounds		hCCR2 IC_{50} (nM)
2 (INCB3344)		5.1
3 (INCB3284)		3.7
4 (INCB8761/PF-4136309)		5.2

involved the replacement of one phenyl ring by a heteroaromatic ring to reduce hydrophobicity (logP) as in INCB3284 (**3**). **3** includes a 6-methoxy-3-pyridyl moiety on side 1 and is a selective and potent CCR2 antagonist with IC_{50} values of 3.7 nM in hCCR2 binding and 4.7 nM in chemotaxis assay. In contrast to INCB3344 (**2**), INCB3284 (**3**) was a substrate for CYP 3A4 and CYP 2D6, but had no inhibitory or inducing effects on the CYP system. The inhibition of the hERG-associated potassium channel was rather low (IC_{50} = 84 μM) [35]. The balanced profile and safety data made INCB3284 (**3**) a promising candidate for phase I and phase II clinical trials, which were unfortunately terminated ahead of schedule [40].

A further clinical candidate from this series, which was tested in osteoarthritis and liver fibrosis, was INCB8761 (a.k.a. PF-4136309) (**4**), which belongs to the group of "inverse" pyrrolidines. Molecular modeling led to a new series of compounds, in which the contacts to the (R)-3-aminopyrrolidine as the main functional

group on side 1 and 3-trifluoromethylbenzoyl aminoacetyl moiety on side 2 in the INCN3284 (**3**) series were reversed [39].

By analogy to the INCB3344 and INCB3284 series, the stereochemistry at the cyclohexyl ring required to be cis. Because previous studies demonstrated that the hydroxyl group and heteroaryl moiety at position 4 of the cyclohexyl ring led to weak hERG blockade and low intrinsic clearance, both structural elements were retained. The 6-methoxy-3-pyridyl moiety, previously present in the lead compound **3**, was replaced by a (pyrimidin-2-yl)-pyridin-2-yl residue in the potent analog INCB8761 (**4**) ($IC_{50} = 5.2$ nM). Compound **4** demonstrated no significant inhibition of other chemokine receptors or any influence on the CYP system. In contrast to the INCB3284 series, (*S*)-configuration in position 3 of the pyrrolidine ring is preferred over the (*R*)-enantiomer [27, 39].

The length of both linkers in compounds **2**, **3**, and **4** corresponds well to the pharmacophore model, where the linker L1 contains 4 carbon atoms and the linker L2 7 carbon atoms.

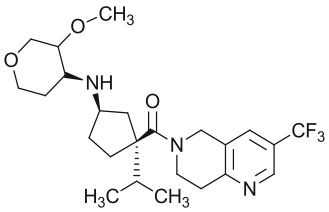
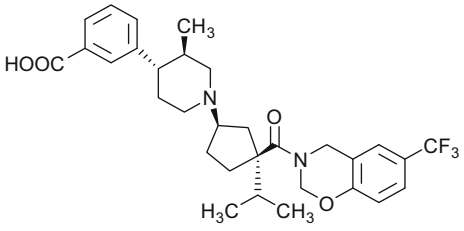
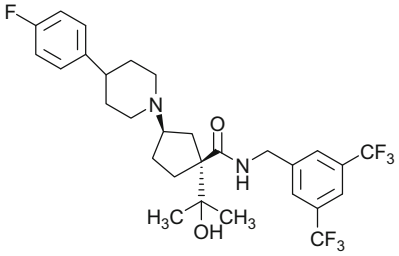
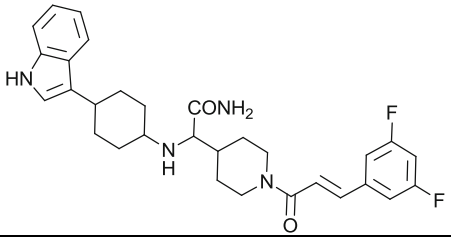
2.4 Piperidine-Based CCR2 Ligands

Merck has disclosed a variety of CCR2 antagonists, which contain a piperidine ring and a cyclopentanecarboxamide substructure. They identified a series of pyrido-annulated piperidines like MK0812 (**5**) that has a tetrahydro-3-trifluoromethyl-1, 6-naphthyridine substructure (Table 2) [41]. This compound contains four chiral centers and is a potent CCR2 antagonist with an IC_{50} value of 5.0 nM and inhibits the chemotaxis with an IC_{50} value of 0.2 nM. MK0812 (**5**) became a clinical candidate in arthritis and multiple sclerosis, but failed in the phase II due to lack of efficacy and was therefore discontinued from the company's pipeline [37, 40].

MK 0483 (**6**) is another potent clinical candidate derived by Merck with an IC_{50} value of 4.0 nM in the CCR2 binding [27]. **6** contains a piperidine and a 1,3-phenoxazine system instead of the tetrahydro-1,6-naphthyridine moiety as in **5** [24]. Further structural variations led to compound **7** not only with a binding affinity of 1.3 nM but also with a potent hERG inhibition ($IC_{50} = 54$ nM). To minimize effects on the hERG channel, the 4-fluorophenyl substituent in the R1 moiety was replaced by diverse more polar aryl and heteroaryl residues. The lowest inhibition of the hERG channel was observed after the introduction of a carboxylic acid in position 3 of the phenyl substituent, unfortunately the CCR2 binding also decreased in similar range [42]. A benzylamide incorporated in **7** is also a promising common structural element of Merck's spirocyclic CCR2 antagonists **13** and **14** (see Sect. 2.6).

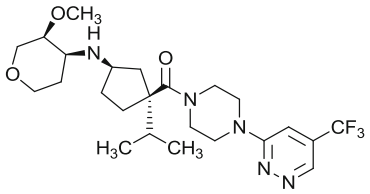
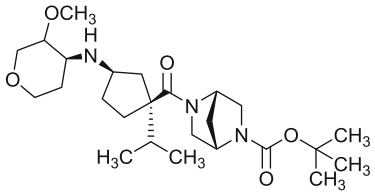
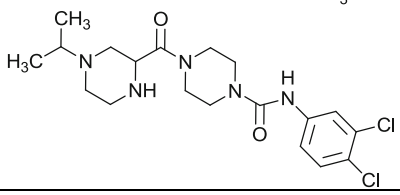
Further development of piperidine-based CCR2 ligands led to a series exemplified by compound **8**. The potent CCR2 antagonist **8** includes a heteroaromatic system of an indole, representing R1, a central core with a cyclohexyl and a piperidine ring and a *trans*-configured cinnamide instead of the benzamide as in 7. In respect to

Table 2 CCR2 antagonists with piperidine and cyclopentancarboxamide substructure, inhibitory effects on the CCL2 binding to the CCR2 receptor

Compounds		hCCR2 IC_{50} (nM)
5 (MK0812)		5.0
6 (MK0483)		4.0
7		1.3
8		12 (*trans*) 740 (*cis*)

cyclohexane stereochemistry, the *trans*-substituted compounds were more active in the CCR2 binding (IC_{50} (hCCR2) = 12 nM) than the cis-configured derivatives (IC_{50} (hCCR2) = 240 nM). It was shown that two substituents in *meta* or *para* position provided the highest CCR2 affinity. Although the in vitro hERG binding of **8** was rather high (IC_{50} = 8 μM), an influence on hemodynamic parameters in a guinea pig model was not observed. Compound **8** also reached animal studies in an inflammation model (thioglycollate-induced peritonitis) [43].

Table 3 CCR2 antagonists with piperazine structure, inhibitory effects on CCL2 binding to human CCR2 receptor

Compounds		hCCR2 IC_{50} (nM)
9 (PF-4254196)		8.1
10		2.9
11		3.5

2.5 *Piperazine-Based CCR2 Ligands*

The replacement of the trifluoromethyl naphthyridine group in MK0812 (**5**) by a (trifluoromethyl pyridazinyl)piperazine moiety led to a new series of piperazine-based CCR2 antagonists. PF-4254196 (**9**) is a potent ligand of the CCR2 receptor (IC_{50} = 8.1 nM) without any cardiovascular liabilities (IC_{50} (hERG) = 31.3 μM) (Table 3) [34]. Similar to Merck's piperidines MK0812 (**5**) and MK0483 (**6**), piperazines **9** and **10** also include a cyclopentane core with an amino substituent in position 3 and a carboxamide and isopropyl substituent in position 1. The development of PF-4254196 (**9**) started with modifications of the spacer length between the cyclopentane carboxamide and the trifluoromethyl containing aryl residues in existing series of CCR2 ligands. Prior compounds included a trifluoromethyl-substituted pyridine but showed a significant hERG inhibition. To eliminate the cardiovascular risk modifications of both, the side 1 tetrahydropyran ring and side 2 heterocycle were explored. Based on former SAR studies, a substitution of side 2 with more polar and/or potential π–π-stacking residues was expected to be well tolerated [34].

The introduction of a pyridazine ring led to PF-4254196 (**9**), which displayed a significantly better CCR2/hERG index and selectivity than the corresponding pyridine-containing compound. Pyridine-based compounds were also reported to be dual CCR2 and CCR5 antagonists [38].

Further modifications led to a methano-bridged piperazine derivative **10** (2,5-diazobicyclo[2.2.1]heptane). Although **10** contains a Boc (*tert*-butoxycarbonyl) moiety instead of an aryl or heteroaryl ring, it showed improved CCR2 affinity (IC_{50} = 2.9 nM) [44].

A further series of potent CCR2 ligands contains an aromatic ring on side 1 and a second terminal piperazine ring on side 2 connected via a carbonyl linker. The prototype of this series **11** demonstrated a high binding affinity to the CCR2 receptor (IC_{50} = 3.5 nM) and a significant reduction of hERG activity compared to methylene-linked subseries and other heterocycles at the side 2. In the hERG assay, a 10,000-fold selectivity for the CCR2 receptor over hERG was observed. Compound **11** did not interact with other chemokine receptors except with the CCR5 receptor (IC_{50} = 22 nM) and can therefore be considered as a dual antagonist with the CCR2 receptor preference [45–47].

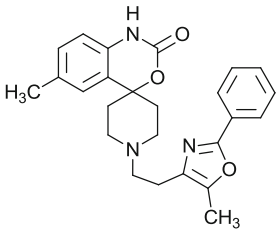
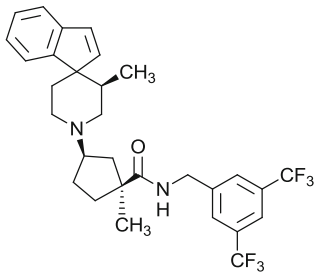
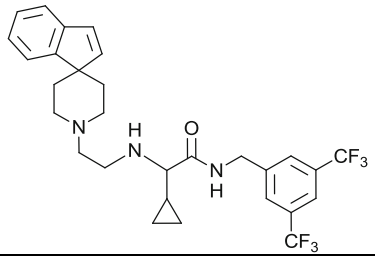
2.6 Spiropiperidine-Based CCR2 Ligands

A prominent example of the spiropiperidine class of CCR2 inhibitors, RS504393 (**12**), was reported by Roche/Iconix. The central structural element of this class included a tertiary amine in a benzannulated piperidine ring system and an orthogonal relationship between the 3,1-benzoxazin-2-one and the piperidine ring, caused by the spirocyclic connection of the rings (Table 4). Another important aspect is the hydrogen binding potential of the urethane moiety and the restriction to small substituents at the benzoxazine heterocycle. SAR studies led to RS504393 (**12**) (IC_{50} = 89 nM) as the most affine compound of this benzoxazine class. The SARs of spiropiperidines were extensively investigated. In these spirobenzoxazine systems, the position 4 of the piperidine is disubstituted by a spiro-phenyl urethane system. Only piperidines or linear alkyl chains were accepted by the CCR2 receptor, other substituents were inactive or revealed reduced affinity. This group of compounds is highly selective for the CCR2 receptor [48].

Site-directed mutagenesis of acidic residues Glu291 and Asp284 in the CCR2 receptor to Ala, Asn, or Gln showed the importance of both Glu291 and Asp284 for ligand interactions via hydrogen bonding towards the tertiary amine of the piperidine ring. For this reason, spiropiperidines show an affinity to receptors in which a glutamic acid residue is in a similar position as in the CCR2 receptor [49]. It was also known that spiropiperidines prevent CCL2 binding by occupying the same region in the inter-helical bundle on the extracellular side [48]. In contrast, the CCR2 receptor binding of CCR2 antagonists without a basic amino moiety was not affected by Glu291 mutations [22].

Merck's compound **13** was claimed as a CCR2 antagonist for the potential treatment of inflammatory and rheumatic diseases [49]. This spiro[indene-piperidine] **13** showed IC_{50} values of 1.3 and 0.45 nM in the CCR2 binding and the chemotactic assay, respectively. **13** possessed a high selectivity over other chemokine receptors including the CCR5 receptor (≈500-fold).

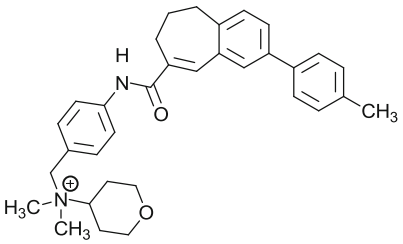
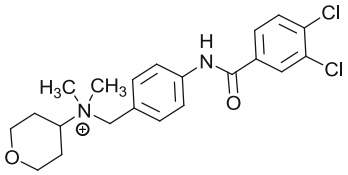
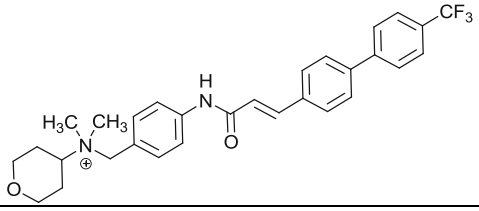
Table 4 CCR2 antagonists with spiropiperidine structure, inhibitory effects on CCL2 binding to human CCR2 receptor

Compounds		hCCR2 IC_{50} (nM)
12 (RS504393)		89
13		1.3
14		4

The tested isomer **13**, shown in Table 4, was found to be the only active stereoisomer of the four possible stereoisomers. The series of spiropiperidines similar to **13** demonstrated that the presence of methyl groups in position 3 of the 1,3-disubstituted cyclopentane ring and in the piperidine ring is crucial for the high affinity at the human and mouse CCR2 receptors. The introduction of an additional methyl group in position 4 of cyclopentane instead of the methyl group in position 3 led to a total loss of the CCR2 activity, as the removal of the methyl group in position 3 of the piperidine ring eliminated CCR2 activity in the assay with both human and murine CCR2 receptors [6, 50]. Compared to analogs without a methyl group in position 1 of the cyclopentane ring, the CCR2 binding affinity increased twofold [51]. Compound **13** also belongs to the series of cyclopentyl and cyclobutyl constrained analogs, in which a quaternary carbon substitution of the central cyclopentane ring was preferred for CCR2 binding [37].

The spiropiperidine **14** from Merck contains a tertiary carbon in side 2. The side 1 is represented by a spiro[indene-piperidine] in compounds **13** and **14**, but the cyclopentyl ring of **13** is replaced by an open chain, which includes an additional

Table 5 CCR2 antagonists with quaternary ammonium salt structure, inhibitory effects on the CCL2 binding to the CCR2 receptor

Compounds		hCCR2 IC_{50} (nM)
15 (TAK-779)		27
16 (JNJ17166864)		20
17		10

secondary amine and a cyclopropylmethyl moiety leading to a tertiary carbon atom. The substituent of the tertiary carbon atom was also varied. A *p*-fluorophenyl substituent at that position led to Merck's "compound 26", a dual CCR2 and CCR5 receptor antagonist, which is, apart from this substituent, identical to the cyclopropylmethyl derivative **14**. In contrast to the *p*-fluorophenyl derivative, the cyclopropyl derivative **14** was selective for the CCR2 receptor, showing an IC_{50} value of 4 nM and promising pharmacokinetic properties [22, 23]. In both **13** and **14**, the aromatic residue R1 is represented by an indene and linked to the central tertiary amine via a 2-carbon linker. The size of linker L2 is broadly based, including 2 atoms in **12,** 6 in **13**, and 7 in **14**.

2.7 Quaternary Ammonium Salts

Potent CCR2 receptor antagonists that contain a quaternary ammonium salt have also been reported (Table 5). The quaternary ammonium moiety of these compounds is expected to form an ionic interaction with Glu291 in the binding pocket of the CCR2 receptor [27]. Developments of these types of CCR2 antagonists started from TAK-779 (**15**, see also Sect. 3.1), first developed as a CCR5 antagonist and later found to have also significant CCR2 affinity (IC_{50} = 27 nM) [40, 52].

Compounds **16** and **17**, both showing promising binding affinities in a ^{125}I-labeled CCL2 assay using a THP-1 cell line, resulted from the systematic modifications of side 2 in **15** [53]. JNJ171668 (**16**), developed by Johnson&Johnson, is a potent candidate with a binding affinity of 20 nM. In line with its quaternary ammonium structure, JNJ171668 (**16**) had poor oral bioavailability, but entered clinical trials for the treatment of allergic rhinitis as a nasal application [40]. The 3,4-dichloro phenyl ring of **16** led to higher binding affinity at the CCR2 receptor than its analogs with other substitution patterns. From a series of different biphenyl-containing compounds, it was evident that the presence of a chloro or bromo group leads to improved binding affinities compared to electron-donating (OMe, Me) and electron-withdrawing groups (CN, CF_3). Structural modifications on the side 2 were also found to be responsible for selectivity related to the interactions with the CCR2 and CCR5 receptor. A different modification on side 2 led to compound **17** with a linker L2 consisting of 9 carbon atoms. **17** contains a biphenyl moiety attached to the 3-position of acrylamide displaying the binding affinity of 10 nM [53]. In comparison with the pharmacophore model, the linker L1 is missing in **15**, **16**, and **17**, whereas R1 is represented by a tetrahydropyran moiety.

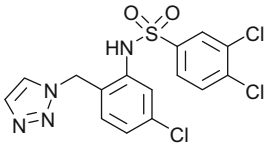
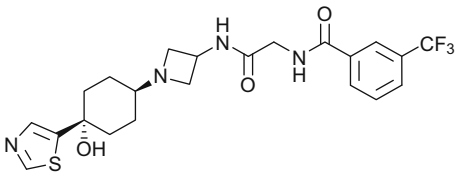
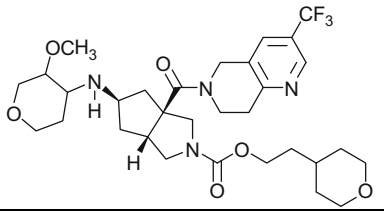
2.8 Latest Structural Developments in CCR2 Ligands

In recent years, new structural classes of CCR2 ligands appeared in the literature, mostly including only small series of compounds. Here, we want to highlight three promising classes, each exemplified by a typical ligand: (1) sulfonamides are represented by **18**, (2) azetidines by **19**, and (3) bicyclic compounds by **20**.

ChemoCentryx and GlaxoSmithKline started the development of sulfonamides as CCR2 ligands [6]. The efforts resulted in the triazolyl-substituted compound as a promising example. Modifications of the substitution pattern led to the trichloro-substituted *N*-phenylbenzenesulfonamide **18** as a potent CCR2 ligand (the GTPγS accumulation assay, $IC_{50} = 10$ nM). **18** inhibited monocyte recruitment but also showed inhibitory effects on the CYP2C19 and CYP2C9 activity (Table 6). The investigation in a thioglycollate-induced peritonitis model for inflammation in a mouse strain with the human CCR2 receptor knocked-in (hCCR2KI mouse) verified the dose-dependent and strain-specific inhibition of monocyte recruitment by **18** [54].

In an analogy to piperidines (see Sect. 2.4) and pyrrolidines (see Sect. 2.3), azetidine-based CCR2 ligands were described. In order to eliminate the zwitterionic character of a piperidine-based amino acid derivative developed by Johnson&Johnson and to increase solubility, a cyclohexylazetidine system was prepared first [31, 55]. Different six- and five-membered heterocycles were introduced in the 4-position of the cyclohexane ring, which reduced the hERG channel activity. Finally, the thiazole derivative **19** was identified as the most potent candidate. A cis orientation of the thiazolyl and the azetidinyl substituents on the cyclohexane ring was found to be essential for the high CCR2 binding affinity [55]. Compound **19** revealed an IC_{50} value of 37 nM in the CCR2 binding assay and did not interact with the hERG channel ($IC_{50} > 50$ μM). A promising cardiovascular safety profile was confirmed in an anesthetized dog safety study. A high selectivity against other chemokine receptors

Table 6 Latest CCR2 antagonists form different structural classes, inhibitory effects on CCL2 binding to the CCR2 receptor

Compounds		hCCR2 IC_{50} (nM)
18		[a]
19		37
20		31

[a] $K_i = 10$ nM

was found, but the pharmacological profile remains to be reported [56]. In 2013, further variations of the heteroaromatic substituents at the *N*-acylglycine moiety were published, which started from **19** as a lead compound. Although various compounds with the high CCR2 affinity, promising functional activity and low hERG channel affinity were identified, none of these compounds displayed a promising pharmacokinetic profile [57].

In 2013, Cai et al. published a novel series of CCR2 antagonists with a bicyclo [3.3.0]octane or bicyclo[4.3.0]nonane scaffold. This class of ligands was designed according to the CCR2 pharmacophore model mentioned above. The first generation was based on a 7-aminobicyclo[3.3.0]octane system [58]. Replacement of the methylene moiety in 3-position by an amino moiety led to the closely related class of 7-amino-3-azabicyclo[3.3.0]octanes. A systematic evaluation of the substituents on the exocyclic amino moiety resulted in **20**, the most promising compound of this series. **20** displayed the high CCR2 affinity ($IC_{50} = 31$ nM) and low cardiovascular risk (hERG $IC_{50} > 50$ μM). The clinical potential of this new candidate will be evaluated after investigation of its in vivo properties [59].

2.9 Binding Poses of Ligands in the CCR2 Receptor

As predicted by computational-based homology modeling studies that included few potent CCR2 ligands, different amino acids were postulated to be essential for the ligand binding to the CCR2 receptor. The most recent studies were based on the

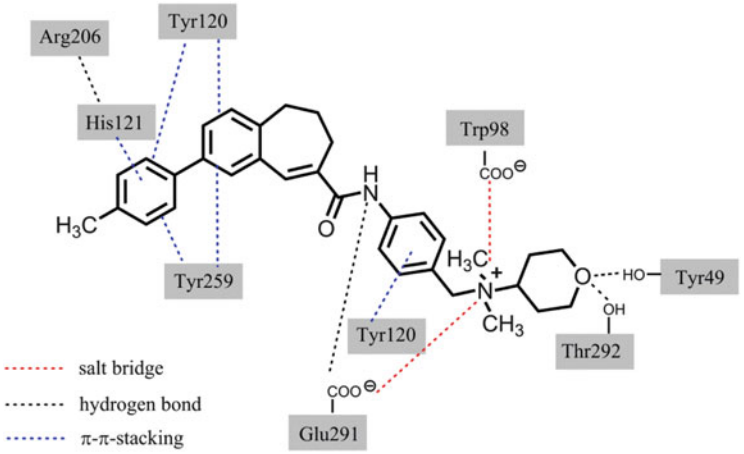


Fig. 3 Schematic presentation of interactions between the CCR2 receptor and TAK-779 (**15**), modified according to [28, 60, 62]

homology modeling, where in 2010 published crystal structure of the closely related CXCR4 receptor served as a template. The CXCR4 structure shows a higher sequence homology as well as a larger binding pocket than the structure of bovine rhodopsin. The binding pocket of the CCR2 receptor is formed by transmembrane domains TM2, TM3, TM5, TM6, and TM7 [28]. Glu291, located on the transmembrane region 7 (TM7), was proposed to be an important anchor residue of various CCR2 ligands, including spiropiperidines [48, 60, 61]. The central basic amine, present in most CCR2 receptor ligands, forms a salt bridge to this conserved acidic residue.

Mutagenesis studies also implied that Tyr120 and His121 might be crucial because of their ability to form hydrogen bonds with endogenous ligands or synthetic molecules. Hydrophobic interactions of the ligands were observed with aromatic residues Tyr49 and Trp98 [28]. The predicted binding site of TAK-779 (**15**) was studied best. The residues of the CCR2 receptor that strongly interact with TAK-779 are shown in Fig. 3.

The most important interaction is the electrostatic interaction of the carboxylate of Glu291 with the quaternary ammonium group of TAK-779. The tetrahydropyran oxygen forms hydrogen bonds with both Tyr49 in TM1 and Thr292 in TM7. The biaryl system on the other site is fixed between the hydroxyphenyl moieties of Tyr120 and Tyr259 and to a lesser extent by His121 via π–π-stacking interactions. His121 also interacts with Arg206 (TM 5), which results in a weaker ligand-histidine interaction. In the CCR5 receptor, His121 is replaced by phenylalanine (see Fig. 5), which cannot form interaction with arginine and therefore adopts an alternative rotameric conformation increasing the π-π-interactions. In case of TAK779, the residues Tyr49, Trp98, Tyr120, and His121 are discussed to form an aromatic cluster contributing to the CCR2 receptor binding [60, 62]. For the binding of the spirocyclic antagonist RS504393 (**12**), Glu291 and Asp284 were identified as hydrogen bond partners for the tertiary amine within the piperidine

ring [48, 49]. Expectedly, the binding of the CCR2 antagonists devoid of basic amine was not affected by Glu291 mutations [22, 23].

2.10 Conclusion

Altogether diverse compounds with high affinity to the CCR2 receptor have been identified. A few CCR2 antagonists were investigated in clinical trials. Up to now, CCR2 antagonists have not shown promising clinical efficacy in inflammatory diseases as presumed from preclinical models. Whether this failure is a result of wrong target selection, off-target effects or poor drug-like properties of the small-molecule antagonist remains to be elucidated [27, 37, 41].

3 CCR5 Ligands

The unique opportunity to study the impact of CCR5 receptor antagonists by exploiting the CCR5Δ32 bp polymorphism as well as its function as a co-receptor for the HIV-host cell fusion brought the CCR5 receptor into focus of many pharmaceutical companies. There are various CCR5 antagonists reported in the literature so far. The CCR5 receptor ligand maraviroc (**1**, Celsentri®, UK-427,827) (Fig. 1) developed by Pfizer is the only CCR5 ligand approved for the treatment of confirmed R5-tropic HIV-1 infection by the FDA and EMEA [2]. On account of this, many further investigations have already been undertaken, starting from maraviroc (**1**) as a lead compound.

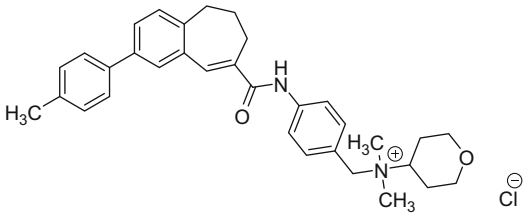
The intention of this chapter is to summarize the SARs of various CCR5 antagonists focusing on CCR5 antagonists derived from TAK compounds (Takeda), maraviroc, and related tropane-based CCR5 ligands. Several different aspects such as antiviral activity, CYP inhibition, leading to several drug-drug interactions and severe adverse effects will be discussed [65, 66]. Inhibition of the hERG K^+-channel is a common challenge in developing CCR5 selective ligands due to the basic amine, which is required for the interaction with Glu283 of CCR5 receptor [29, 65, 66]. Therefore, the affinity to the hERG K^+-channel [67] and the oral bioavailability, which significantly influence the CCR5 ligand development, will be discussed.

3.1 CCR5 Ligands Developed by Takeda Inc

3.1.1 Quaternary Ammonium Salts and Tertiary Amine-Based CCR5 Antagonists

Takeda Pharmaceutical Company has set themselves the task of creating a new class of antihuman immunodeficiency virus 1 (HIV-1) entry inhibitors. One way to

Fig. 4 TAK-779 (**15**)



inhibit HIV-1 replication is to prevent the viral entry into the target cell. The potential of this approach is shown by T20, a peptide that prevents the conformational change in the viral glycoprotein gp41 that drives membrane fusion [68]. Therefore, Takeda has designed several compounds which were based on hits of a high-throughput screening (HTS). The most promising compound resulting from these hits was TAK-779 (**15**) (Fig. 4).

TAK-779 (**15**) antagonizes the binding of CCL5 to CCR5-expressing Chinese hamster ovary (CHO) cells completely at a concentration of 100 nM and showed an IC_{50} value of 1.4 nM. Moreover, **15** was shown to block membrane fusion of HIV-1 at nanomolar concentrations. The binding of CCL3 and CCL4 to the CCR5-expressing cells was also blocked with IC_{50} values around 1.0 nM. Although TAK-779 inhibited the binding of [^{125}I]-CCL2 to CCR2 in CHO/CCR2 cells, its IC_{50} value for CCR2 receptor (IC_{50} = 25 nM) was approximately 20-fold higher than that for CCR5 receptor [52]. The sequence homology between CCR5 and CCR2 receptors is 76% [69], which might explain the dual antagonistic character.

Molecular modeling and mutagenesis studies have shown that the active site of the CCR5 receptor is very hydrophobic with multiple aromatic residues forming a tight binding pocket [70]. The benzene ring of the benzo[7]annulene moiety of **15** was observed to interact with aromatic side chains of Tyr108 and Trp248 via a T-shaped π–π-stacking. Additionally Tyr108 forms a hydrogen-bond interaction between the phenolic OH group and the carbonyl moiety of **15**. Strong hydrophobic interaction between the *p*-tolyl group and Ile198 on TM5 accompanied by some weaker interactions of **15** with Thr195, Ile198, Phe109, Trp248, and Tyr251 were also found. The limited ionic interaction between the quaternary ammonium moiety of **15** and Glu283 was caused by the steric shielding of the positively charged center (Fig. 5) [63].

Due to the fact that **15** inhibits the CCR5 receptor, an anti-R5 HIV-1 assay was performed. The measured effective concentrations in the anti-fusion assay were 1.2 nM (EC_{50}) and 5.7 nM (EC_{90}) [71]. It was additionally shown that TAK-779 did not interact with CCR1, CCR3, or CCR4 receptors. The quaternary ammonium moiety of TAK-779 led to a good binding affinity, but poor oral bioavailability, which required further optimization.

In order to develop an active CCR5 antagonist with a reasonable oral bioavailability, derivatives of tertiary amines were investigated. The tertiary amine **21**, derived from TAK-779 by removing one CH_3 group, resulted in decrease of CCR5 affinity but increased oral bioavailability. In order to enhance the CCR5, affinity modifications of the [7]annulene ring were undertaken [72]. The exchange

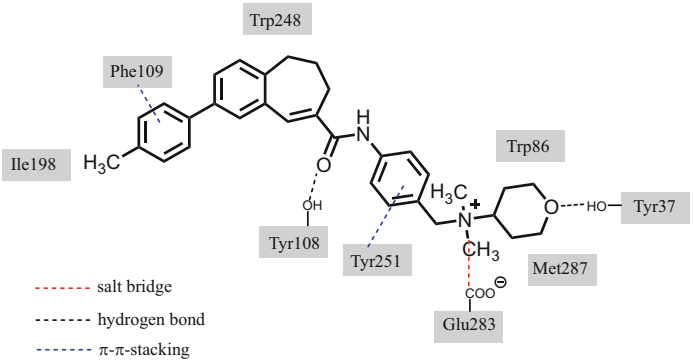
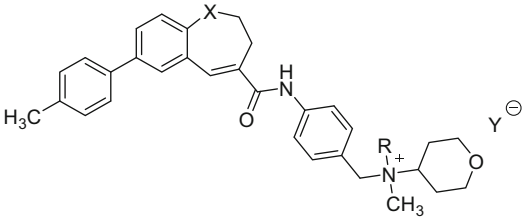


Fig. 5 Schematic presentation of interactions between CCR5 receptor and TAK-779 (**15**), modified according to [63, 64]

Table 7 CCR5 affinities of quaternary ammonium salts **15** and **25** and the tertiary amines **21**–**24**, **26**, and **27** [72, 73, 75] and inhibitory effects on [^{125}I]-CCL5 binding to CCR5-expressing CHO cells

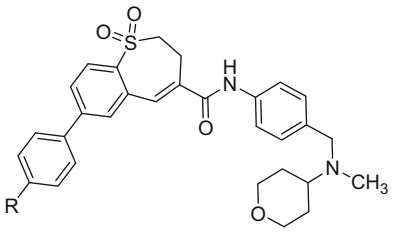


Compounds	*X*	*R*	*Y*	CCR5 IC_{50} (nM)
15	CH_2	CH_3	Cl	1.4
21	CH_2	–		950
22	S	–		800
23	SO	–		300
24	SO_2	–		200
25	O	CH_3	Cl	1.4
26	O	–		530
27	NCH_3	–		130

at the 5-CH_2-moiety of the benzo[7]annulene of TAK-779 (**15**) by a S-atom (**22**) did not significantly increase CCR5 receptor affinity [73]. The introduction of a sulfoxide (**23**) or a sulfone (**24**) led to a slightly increased affinity [74]. The exchange of the 5-CH_2-group by an O-atom resulted in the benzoxepine **25** with high CCR5 affinity, which could not be retained in the tertiary amine **26**. The highest affinity of the tertiary amines was found for the 1-methyl-1-benzazepine **27** (IC_{50} = 130 nM) [72]. Because the compounds **24** and **27** possess high oral bioavailability (>50%) in rats [73], further variations of the benzothiepine-1,1-dioxide and the 1-benzazepine cores were envisaged (Table 7).

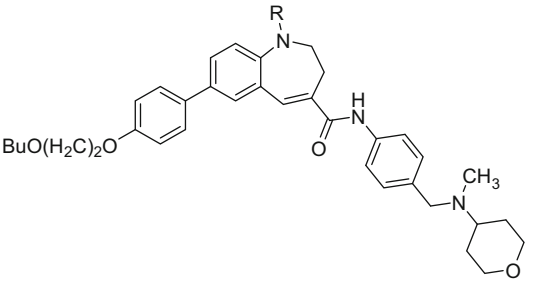
In order to enhance the CCR5 receptor affinity, modifications of the *p*-methyl group of **24** were investigated (Table 8). Replacement of the methyl group by an ethyl substituent (**28**) led to a 3-fold increased CCR5 receptor affinity, which was

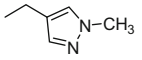
Table 8 Variation of the *p*-CH_3 group [75], inhibitory effects on the [^{125}I]-CCL5 binding to CCR5-expressing CHO cells



Compounds	*R*	IC_{50} (nM)
24	H_3C-	200
28	Et−	60
29	PrO−	35
30	$BuO(CH_2)_2O-$	27

Table 9 Variation of the N-substituent



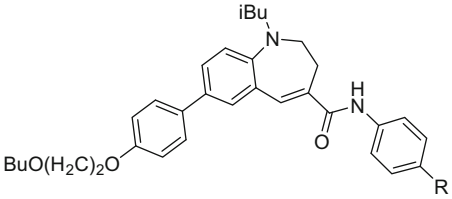
Compounds	*R*	CCR5 IC_{50} (nM)[a]	Membrane fusion IC_{50} (nM)[b]
31	Et	5.6	1,000
32	Pr	3.5	54
33	*i*-Bu	3.6	1.7
34		4.5	150
35	Bn	5.3	2.3
36		2.7	1.2

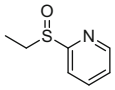
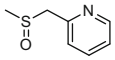
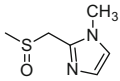
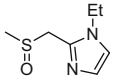
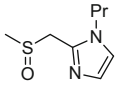
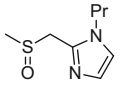
[a]Inhibitory effects on [^{125}I]-CCL5 binding to CCR5-expressing CHO cells
[b]Inhibition of membrane fusion [75]

further increased by introduction of alkoxy groups (compounds **29** and **30**) [75]. The butoxyethoxy derivative **30** was chosen for further optimization.

The combination of the butoxyethoxy group with a 1-benzazepine scaffold led to the next series of CCR5 antagonists. Homologation of the N-methyl group to an N ethyl group (**31**) resulted in an increased CCR5 affinity, but low inhibition of HIV-1 envelope-mediated membrane fusion (Table 9). Introduction of propyl (**32**), isobutyl (**33**), benzyl (**35**), and methylpyrazolyl (**36**) residues increased the inhibitory activity, whereas the cyclopropylmethyl group (**34**) resulted in a remarkable drop of inhibitory activity [75].

Table 10 Introduction of a sulfoxide, inhibitory effects on the binding of [^{125}I]-CCL5 to CCR5-expressing CHO cells



Compounds	*R*	CCR5 IC_{50} (nM)
37		450
38		250
39		67
40		14
41		6.6
42		4.3
(*S*)-**42**		1.9

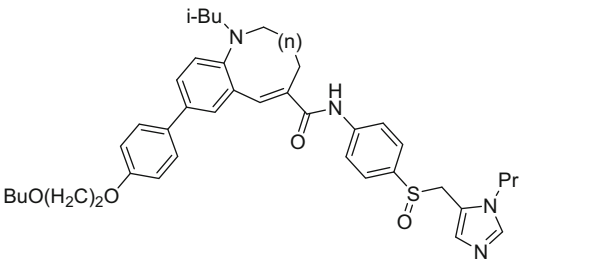
3.1.2 1-Benzazepine and 1-Benzazocine-Based CCR5 Ligands

Compounds **37–42** were synthesized to examine the effect of various sulfoxides on the CCR5 affinity (Table 10). The methylimidazolyl-sulfinyl derivative **40** led to increased CCR5 receptor affinity. Elongation of the alkyl substituent to an ethyl (**41**) or propyl (**42**) moiety led to increased CCR5 affinity. The enantiomer (*S*)-**42** was found to be more potent than the (*R*)-enantiomer (*R*)-**42** [72].

Expansion of the seven-membered azepine ring of (*S*)-**42** to an azocine ((*S*)-**43**), azonine ((*S*)-**44**), and azecine ring ((*S*)-**45**) led to a series of potent compounds (Table 11) [72].

Due to the high CCR5 affinity, virus fusion inhibition, and oral bioavailability, TAK-652 ((*S*)-**43**) became a promising HIV-1 entry inhibitor for clinical studies. It was shown that TAK-652 inhibited the binding of CCL5 (IC_{50} = 3.1 nM), CCL3,

Table 11 Variation of ring size



Compounds	*n*	CCR5 IC_{50} (nM)[a]	Membrane fusion IC_{50} (nM)[b]
(*S*)-**42**	0	1.9	1.0
(*S*)-**43** (TAK-652)	1	3.1	0.1
(*S*)-**44**	2	6.8	0.48
(*S*)-**45**	3	67	–

[a]Inhibitory effects on the binding of [^{125}I]-CCL5 to CCR5-expressing CHO cells
[b]Inhibitory effects on the binding of HIV-1 envelope-mediated membrane fusion

and CCL4 (IC_{50} = 2.3 nM) to the CCR5 receptor and also blocked CCL2 binding (IC_{50} = 5.9 nM) to the CCR2 receptor. In further tests, the inhibitory effect on the fusion between the HIV-1 envelop protein and the cell membrane was investigated with TAK-652 (IC_{50} = 0.1 nM). The replication of all HIV-1 isolates in peripheral blood mononuclear cells (PBMCs) was inhibited by TAK-652 with EC_{50} and EC_{90} values of 0.061 and 0.25 nM, respectively [76].

3.1.3 Propandiamine-Based CCR5 Ligands

Compounds **46–48** represent CCR5 antagonists with entirely different structures. The core structure of **46–48** is characterized by an *N*-(3-piperidinopropyl) carboxamide. The propandiamine substructure has become an important pharmacophore element for the development of CCR5 antagonists (Table 12).

The HTS of the Takeda's compound library led to the discovery of *N*-(piperidinopropyl)carboxamide **46** with low micromolar CCR5 binding affinity. Subsequent optimization resulted in a series of piperidine-4-carboxamides, exemplified by **47**, which had low nanomolar affinity for CCR5 receptors and exhibited high anti-HIV-1 activity [77]. The fast metabolism of **47** stimulated further optimization, which led to the most promising derivative **48** (TAK-220) of this new series (Table 12). It demonstrated high inhibition of the [^{125}I]-CCL5 binding to CCR5-expressing CHO cells (IC_{50} = 3.5 nM), high inhibition for HIV-1 membrane fusion (IC_{50} = 0.42 nM), and also high metabolic stability upon incubation with human hepatic microsomes [78]. A comparison of binding affinities and antiviral activity of TAK-779 (**15**), TAK-652 (**43**), and TAK-220 (**48**) is summarized in Table 13.

Table 12 Inhibitory effects on the binding of [^{125}I]-CCL5 to CCR5-expressing CHO cells

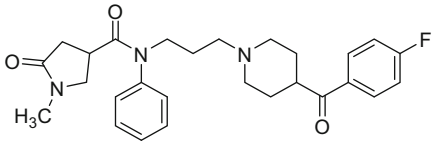
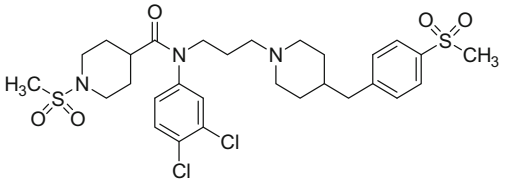
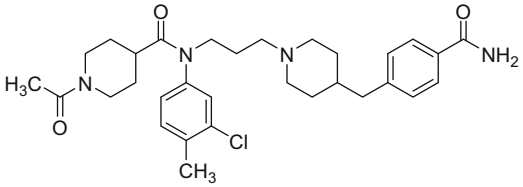
Compounds		CCR5 IC_{50} (nM)
46		1,900
47		2.3
48 (TAK-220)		3.5

Table 13 CCR5 and CCR2 binding affinities of compounds **15** (TAK-779), **43** (TAK-652) and **48** (TAK-220)

Compounds	CCR5 IC_{50} (nM)[a]	CCR2 IC_{50} (nM)[b]	Membrane fusion IC_{50} (nM)[c]
15 (TAK-779)	1.4	27	15
43 (TAK-652)	3.1	5.9	0.1
48 (TAK-220)	3.5	–	0.42

[a]Inhibitory effects (IC_{50}) on the binding of [^{125}I]-CCL5 to CCR5-expressing CHO cells
[b]Inhibitory effects (IC_{50}) on the binding of [^{125}I]-MIC-1 to CCR2b-expressing CHO cells
[c]Inhibition (IC_{50}) of HIV-1 envelope-mediated membrane fusion

The conformational flexibility of TAK-220 is higher than that of TAK-779 due to the higher number of rotatable bonds. Docking into a 3-D homology model of the CCR5 receptor showed that TAK-220 forms a strong salt bridge with Glu283. Mutagenesis studies indicated that the residues Trp86, Tyr108, Trp248, Tyr251, and Met287 (see Fig. 5) are important for TAK-779 binding, but have little effects on TAK-220 binding. However the hydrophobic interaction of TAK-220 with Ile198 is as strong as in case of TAK-779 binding (Fig. 6). The 3-chloro-4-methylphenyl group of TAK-220 is placed in the similar region within the helical bundle between Phe109, Trp248, and Tyr251 as the phenyl group of maraviroc (**1**) [63, 79].

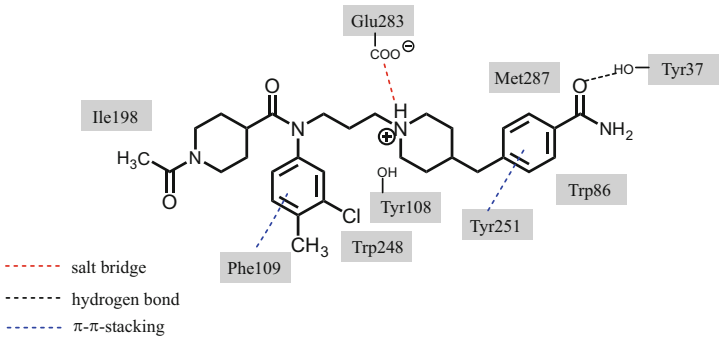


Fig. 6 Schematic presentation of interactions between the CCR5 receptor and TAK-220 (**48**), modified according to [79]

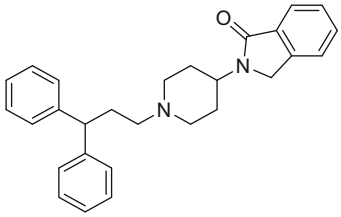
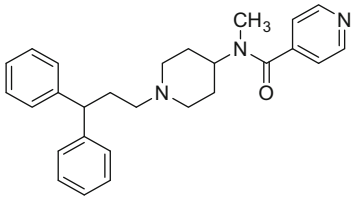
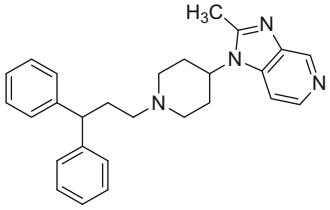
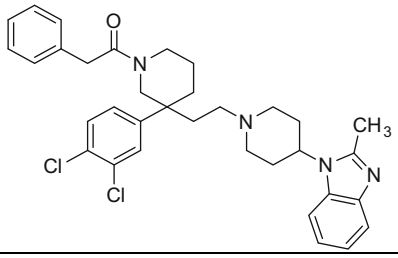
3.2 The Development of Maraviroc and Related Tropane-Based CCR5 Ligands

3.2.1 1-(3,3-Diphenylpropyl)-Piperidinyl and 1-(3-Amido-3-Phenylpropyl)-Piperidinyl-Based CCR5 Ligands

The first 1-(3,3-diphenylpropyl)piperidine-based CCR5 antagonists were found by AstraZeneca and Pfizer by an HTS of their compound libraries [45, 80]. AstraZeneca's screen resulted in two closely related hits, **49** and **50,** and Pfizer's screen in the hits **51** (UK-107,543) and **52** (Table 14).

AstraZeneca's approach focused on the development of CCR5-selective antagonists for the treatment of chronic inflammatory diseases, such as rheumatoid arthritis [81] and inflammatory bowel disease [82]. Compounds **49** and **50** demonstrated similar CCR5 binding affinities in low micromolar range, indicating no advantage of the cyclized N-substituent of **49**. The SAR investigations, in which the substituent R^2 of the acyl group was varied, revealed that neither (hetero)aromatic nor aliphatic groups increased the CCR5 receptor affinity (Table 15). The phenylacetyl derivative **53** was the only compound with potency in the high sub-micromolar range. Introduction of substituents at the *o*- and *m*-position of the phenylacetyl group did not significantly affect the binding affinity. However, an increase in affinity was observed after introduction of polar electron-withdrawing substituents in *p*-position. In particular, sulfamoyl (**55**), *N*,*N*-dimethylsulfamoyl (**56, 57**), and methylsulfonyl (**58**) groups showed nanomolar CCR5 affinities. Replacing the methyl (**56**) with the ethyl group (**57**) at the amide N-atom slightly increased the CCR5 affinity (Table 15). Replacement of the amide substructure by a sulfonamide moiety was shown to be detrimental, whereas the introduction of a urea moiety instead of the amide retained the CCR5 affinity. Compounds **55**, **56**,

Table 14 HTS hits **49**, **50** (AstraZeneca), **51** (UK-107,543), and **52** (Pfizer)

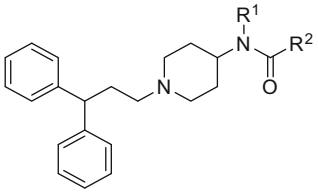
Compounds		CCR5 IC_{50} (nM)
49		1,900[a]
50		2,300[a]
51 (UK-107,543)		400[b]
52		1,100[b]

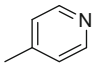
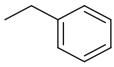
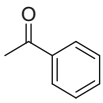
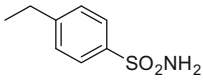
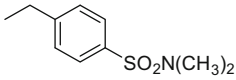
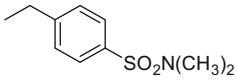
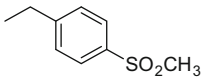
[a]Inhibition of [^{125}I]-CCL5 binding to human CCR5 receptors
[b]Inhibition of CCL4 binding to the human CCR5 receptor in stably expressed HEK-293 cells

and **57** showed no affinity towards CCR1, CCR2b, CCR3, CXCR1, and CXCR2 receptors. The *N*,*N*-dimethylsulfonamide **57** displayed micromolar affinity to muscarinic and serotonergic receptors as well as the hERG channel [45]. Therefore, the methyl sulfone **58** was chosen as the new lead compound for further ligand development.

Next SAR studies around the benzhydryl structure were undertaken. Introduction of one **60** or two **59** fluorine atoms into the *p*-position of the phenyl rings resulted in decreased CCR5 affinity. In contrast, one chlorine atom in *p*-position (**61**) was highly beneficial. Therefore, further substituents in *p*-position of one

Table 15 CCR5 receptor affinities of compounds **49, 50, 53–57**. Inhibitory effects of [^{125}I]-CCL5 to human CCR5 receptors

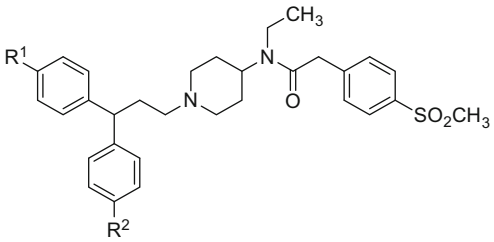


Compounds	R^1	R^2	CCR5 IC_{50} (nM)
49	H_3C–	–	4,100
50	H_3C–		6,100
53	H_3C–		810
54	H_3C–		5,900
55	H_3C–		91
56	H_3C–		46
57	Ethyl–		<10
58	Ethyl–		76

phenyl ring, retaining the other phenyl ring unsubstituted, were investigated. Compounds with strongly electron-withdrawing substituents such as trifluoromethyl (**62**), cyano (**64**), and methylsulfonyl (**66**) were highly potent, but also the methoxy derivative **65** demonstrated high CCR5 affinity (Table 16). Electronic effects alone are not sufficient to explain the SAR. The weakly potent difluoro (**59**) and 4-fluoro (**60**) compounds as well as the methylsulfonyl (**66**) derivative demonstrated sufficient metabolic stability and a good pharmacokinetic (PK) profile. The potential of the fluorine atoms to reduce oxidative metabolism on the phenyl rings of the diphenylpropyl moiety indicated a possible approach to improve oral bioavailability in this series of CCR5 ligands [47].

The (*S*)-enantiomer of the methylsulfonyl derivative (*S*)-**66** was found to be twice as potent as the racemate **66.** Moreover the enantioselective synthesis of (*S*)-**66** was used to prepare several analogs with various substituents at the second phenyl ring.

Table 16 IC_{50} values of 1-(3,3-diphenylpropyl)-piperidine derivatives **58–66** with various substituents at the phenyl residues. Inhibition of CCL3 binding to the human CCR5 receptor

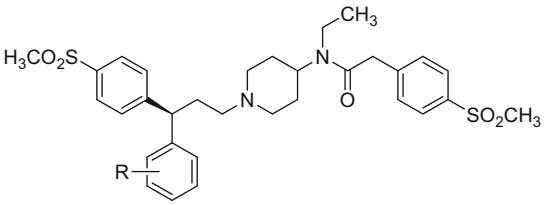


Compounds	R^1	R^2	CCR5 IC_{50} (nM)
58	H–	H–	18
59	F–	F–	780
60	F–	H–	310
61	Cl–	H–	8.5
62	F_3C–	H–	2.3
63	H_3CO_2C–	H–	7.1
64	CN–	H–	<1.0
65	H_3CO–	H–	6.3
66	H_3CSO_2–	H–	1.7

Since fluorine atom in *p*-position of the second phenyl moiety led to dramatic loss of the CCR5 affinity, the *m*-position was addressed for the introduction of the fluoro substituent (**67**). **67** displayed increased CCR5 affinity, but also fast clearance and short half-life. This could be improved by introduction of halogen atoms at both *m*-positions (Table 17). The 3,5-*di*fluoro (**69**), 3-fluoro (**67**), and 5-chloro (**68**) analogs showed favorable pharmacokinetic profiles. The 3,5-difluoro derivative **69** displayed no affinity towards CCR1, CCR2b, CCR3, CXCR1, and CXCR2 receptors and other human G-protein-coupled receptors (human M_1, M_2, and 5-HT_{2A} receptor). Unfortunately, an inhibition of CYP 2D6 (1.6 μM) and hERG ion channel binding (7.3 μM) were detected [46].

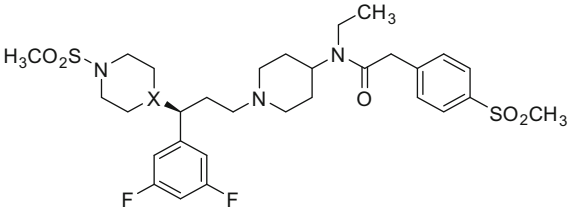
In order to reduce cardiotoxicity, the benzhydryl part of the molecule was further modified. SAR investigation clearly indicated the requirement of one phenyl substituent, whereas the replacement of the second phenyl ring by other substituents was tolerated. Introduction of a piperazine and C-atom-linked piperidine ring led to compounds with reduced lipophilicity, promising CCR5 affinity and decreased hERG ion channel binding (Table 18) [83]. The piperazine derivative **71** showed only moderate bioavailability in dogs and very fast plasma clearance in rats, whereas the C-linked piperidine with the methylsulfonyl substituent at the N-atom demonstrated good bioavailability in both species with high selectivity over CYP 1A1, 2C9, 2C19, 2D6, and 3A4 enzymes. Therefore **72** (AZD5672) was selected as drug candidate for the treatment of rheumatoid arthritis (RA). The development of AZD5672 (**72**) was terminated in a phase IIb study with RA

Table 17 CCR5 affinity of compounds **66–70** with various substituents in the *m*-position of the second phenyl ring. Inhibition of [^{125}I]-CCL3 binding to the human CCR5 receptor



Compounds	*R*	CCR5 IC_{50} (nM)
(*S*)-**66**	H–	0.76
(*R*)-**67**	3-F–	0.22
(*R*)-**68**	3-Cl–	1.0
(*R*)-**69**	3,5-*di*-F–	0.32
(*R*)-**70**	3-F–, 5-Cl–	1.1

Table 18 Variations (**71**, **72**) of the second phenyl ring



Compounds	*X*	CCR5 IC_{50} (nM)[a]	hERG inhibition IC_{50} (nM)[b]
71	N	3.7	>32,000
72 (AZD5672)	CH	0.26	24,000

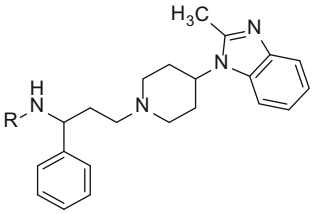
[a]Inhibition of [^{125}I]-CCL3 binding to the human CCR5 receptor
[b]The concentration required to inhibit binding of [^{3}H]dofetilide binding to hERG stably expressed on HEK-293 cells

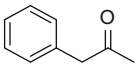
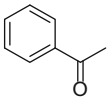
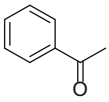
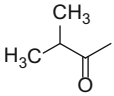
patients, due to absence of statistically significant effects of AZD5672 on symptoms of RA [83].

Pfizer's approach focused on the development of CCR5 selective ligands for the treatment of HIV-1 infection [84, 85]. Compounds **51** and **52** showed weak CCR5 binding affinity, antiviral activity could not be detected, and, moreover, high affinity to the CYP 2D6 enzyme was found (Tables 14 and 19) [80].

The replacement of the imidazopyridine structure, responsible for the interaction with CYP 2D6 by coordination of the pyridine N-atom to the heme iron, led to benzimidazole **73**. Compound **73** showed potent inhibition of CCL4 binding and much weaker CYP 2D6 inhibition, but still no antiviral activity (Table 19). In order

Table 19 The CCR5 receptor affinity and antiviral activity of compounds **51, 52,** and **73–77**



Compounds	*R*	CCR5 IC_{50} (nM)[a]	Antiviral activity IC_{50} (nM)[b]
51	–	400	–
52	–	1,100	–
73		4	–
74		100	740
75		45	210
(*S*)-**75**		13	190
76		50	700
77		40	75
(*S*)-**77** (UK-347,503)		20	73

[a]Inhibition of the [^{125}I]-CCL4 binding to the human CCR5 receptor
[b]Antiviral activity determined against HIV-Bal in PM-1 cells [80]

to increase the polarity, one of the phenyl groups of the diphenylmethyl moiety was replaced by an amide bearing substructure already found in compound **52**. Amides **74–77** inhibited the CCL4 binding but, more interestingly, moderate levels of antiviral activity determined against HIV-Bal in PM-1 cells [80, 86] were found (Table 19).

The benzamide **75**, the isobutyramide **76**, and the cyclobutanecarboxamide **77** were found to be the most active antiviral compounds in this series. The data of CCL4 inhibition and antiviral activity do not correlate, indicating that the binding domains of HIV gp120 and CCL4 are distinct and separate. In order to determine the eutomers within the amide series, the benzamide **75** and the cyclobutanecarboxamide **77** were synthesized stereoselectively. The (*S*)-enantiomers (*S*)-**75** and (*S*)-**77** had higher CCR5 affinity and antiviral activity than the (*R*)-enantiomers. Compound UK-347,503 ((*S*)-**77**) also showed decreased affinity to the CYP 2D6 enzyme and was chosen as a lead compound for further CCR5 antagonist development [80].

3.2.2 Tropane-Based CCR5 Ligands

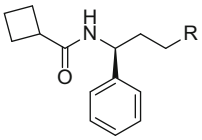
Compounds with affinity to the CYP 2D6 enzyme have a basic amino group in 5–7 Å distance to a possible site of oxidation. The basic amine is interacting with Asp301 of the enzyme [87, 88]. In order to avoid CYP 2D6 affinity a series of analogs of **77** with a modified piperidine ring was designed.

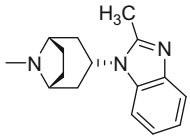
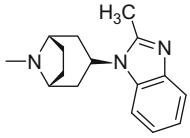
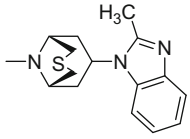
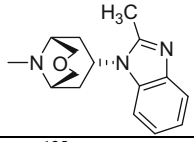
The benzimidazole derivatives *exo*-**78** and *endo*-**78** demonstrated increased inhibition of viral replication (Table 20) combined with reduced CYP 2D6 affinity. The similar activity of the *exo*- and the *endo*-isomers is caused by different orientations of the bridged piperidine rings. The benzimidazole forces the isomer *endo*-**78** into a boat conformation, well overlapping with the chair conformation of *exo*-**78** [1]**.** All compounds **78–80** demonstrated potent antiviral activity against clinically relevant CCR5-tropic viruses, but failed in safety screenings due to high inhibition of the hERG ion channel [89]. Therefore the next aim was to obtain selectivity against the hERG channel.

The first approach to overcome hERG affinity was driven by the exploration of the prodrug concept of compound **81**. Compound **81** demonstrated high bioavailability and high hERG binding but was rapidly oxidized to the highly selective primary metabolites tetrahydropyran S-oxide **82** and S,S-dioxide **83, 82** and **83** displayed high inhibition of cell-cell fusion without any binding to the hERG channel at 10 μM in in vitro assays (Table 21). However, the bioavailability of metabolites **82** and **83** after *p.o.* administration of **81** to rats was lower than 10%. Gut wall metabolism and excretion by the liver were suggested to be responsible to the failure of **81** as oxidizable prodrug [74].

Because the first strategy to overcome hERG affinity by a prodrug concept failed, the second strategy focused on the modification of the basicity and steric environment of the central amino moiety and alteration of the orientation and substitution patterns of the aromatic rings in lead compound **78**. In the oxagranatane *exo*-**80** (Table 20), the basicity of the central amino group is reduced to pK_a 6.0 compared to pK_a 7.8 of e*xo*-**78**. However, the hERG channel affinity was not

Table 20 CCL4 inhibitory activity and antiviral activity of piperidine analogs [1]

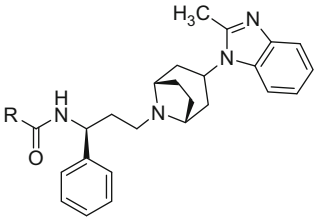


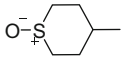
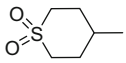
Compounds	*R*	CCR5 IC_{50} (nM)[a]	Antiviral activity IC_{90} (nM)[b]	hERG channel inhibition[c]
exo-**78**		2	13	80% at 300 nM
endo-**78**		6	3	99% at 300 nM
79		21.5	–	–
exo-**80**		9.0	–	70% at 300 nM

[a]Inhibition of the [^{125}I]-CCL4 binding to the human CCR5 receptor
[b]Antiviral activity determined against HIV-Bal in PM-1 cells
[c]Inhibition (%) of [^{3}H]dofetilide binding to hERG stably expressed on HEK-293 cells

reduced, which suggested that the basic center itself is not essential for hERG binding [90]. Docking of **78** into a hERG channel model indicated a lipophilic interaction of the phenyl ring of the benzimidazole moiety with the hERG channel residues. In order to inhibit this overlap, a triazole moiety (**84**) instead of the benzimidazole ring (**78**) was introduced, which dramatically decreased the hERG affinity. The introduction of an isopropyl side chain at the triazole motif (**85**) increased the antiviral activity, but the added lipophilicity increased the affinity to the hERG ion channel as well. The cyclobutyl group of the amide **78** was shown to overlap nicely with the lipophilic binding pocket of the hERG channel. In order to interrupt this interaction, polar fluorinated groups were introduced (**86**, **1**). The 4,4-difluorocyclohexyl derivative **1** does not show any binding to the hERG channel, even at a concentration of 1,000 nM. Combined with low nanomolar antiviral potency (Table 22) [89], a broad anti-R5 HIV-1 spectrum and no inhibition of CYP 1A2, 2C9, 2C19, 3A4, and 2D6 enzymes compound **1** were characterized

Table 21 Compounds **82** and **83** as active metabolites of **81**



Compounds	*R*	Membrane fusion IC_{50} (nM)[a]	hERG inhibition IC_{90} (nM)[b]
81		0.2	740
82		0.2	>10,000
83		0.2	>10,000

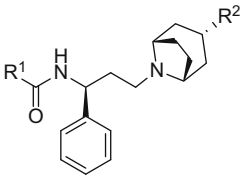
[a]Inhibition gp160 fusion
[b]Inhibition of [^{3}H]dofetilide binding to hERG stably expressed on HEK-293 cells

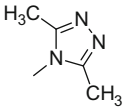
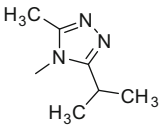
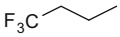
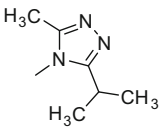
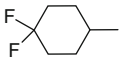
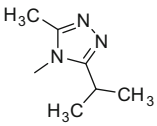
as an inverse agonist of CCR5 receptors, stabilizing the receptor in the inactive conformation [91].

Maraviroc (**1**, UK-427,857) was the product of a long optimization process leading to the first CCR5 ligand approved for the treatment of confirmed R5-tropic HIV-1 infection on the market [2]. The use of maraviroc for the HIV-1 therapy is currently complicated by the growing number of maraviroc-resistant HI-virus strains (MVC^{RES}) [92–94], which makes the ligands with an improved resistance profile desirable.

The crystal structure of the CCR5-maraviroc complex, reported in 2013, shows the binding of the ligand at the bottom of a pocket formed by residues from helices TM1, 2, 3, 5, 6, and 7. The tropane N-atom is protonated and forms a salt bridge with Glu283. The NH moiety of the amide forms a hydrogen bond with the phenolic group of Tyr251 (Fig. 7). The length of the propyl chain between the two N-atoms correlates with the positions of Glu283 and Tyr251 in the receptor. The fluorine atoms in the cyclohexane ring form two hydrogen bonds with Thr195 and Thr245. The phenyl group interacts with five aromatic residues, Tyr108, Phe109, Phe112, Trp248, and Tyr 251 in the binding pocket. The interaction of the benzene ring with Trp248 is believed to prevent the activation-related motion of the receptor, which underlines the inverse agonist character of maraviroc (see Fig. 7) [95]. Compared to the CXCR4/IT1t structure [30], the binding site of maraviroc (**1**) was found to be deeper, without any contact to extracellular loops. The availability of the X-ray crystal structure of the CCR5 receptor will help to promote the development of novel potent CCR5 ligands with optimized properties.

Table 22 Binding data of compounds **1**, **84–86**



Compounds	R^1	R^2	Antiviral activity IC_{50} (nM)[a]	hERG inhibition[b]
84			13	10% at 100 nM
85			8	14% at 100 nM 30% at 300 nM
86			14	14% at 300 nM
1 (UK-427,857, maraviroc)			2	0% at 300 nM 0% at 1,000 nM

[a]Inhibitory effect on HIV-Bal virus replication of in PM-1 cells
[b]Percentage inhibition of [^{3}H]dofetilide binding to hERG stably expressed on HEK-293 cells

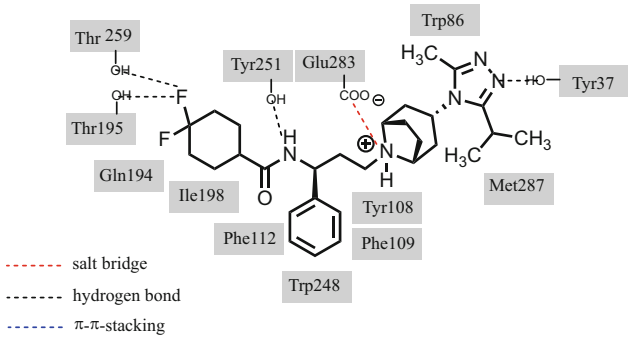
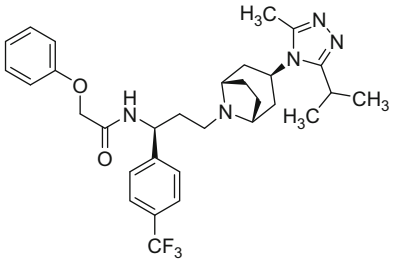
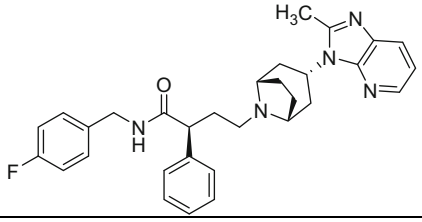


Fig. 7 Schematic representation of interactions between the CCR5 receptor and maraviroc (**1**), modified according to [95]

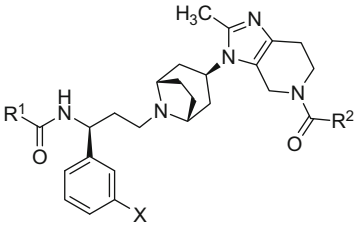
Table 23 CCR5 ligands **87** and **88** developed by lead deconstruction strategy. Inhibition of CCL5-stimulated [^{35}S]-GTPγS accumulation to CCR5-expressing CHO cell membranes

Compounds		CCR5 IC_{50} (nM)
87 TD0444		253
exo-**88**		14

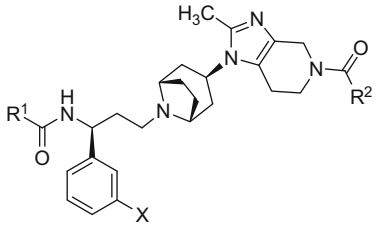
Long and coworkers from the Shanghai Institute of Materia Medica developed the lead compound **1** in more detail by applying lead deconstruction strategy. This approach combines privileged structures of a lead compound with new motifs. Replacement of the difluorocyclohexyl moiety of maraviroc by a phenoxy group and the introduction of the trifluoromethyl group at the *p*-position of the phenyl ring resulted in the moderate CCR5 ligand **87** (TD0444, Table 23). Further improvement of the CCR5 affinity was achieved by introduction of an *exo*-oriented 2-methyl-3*H*-imidazo[4,5-*b*]pyridine-3-yl residue instead of the triazolyl moiety and inversion of the amide substructure, which led to the potent CCR5 ligand **88**, whereas the corresponding *endo*-isomer of **88** is inactive (Table 23) [96, 97].

PF-232798 (**90c**, Table 24) is the follow-up clinical candidate of maraviroc (**1**), currently in phase II clinical studies, evolved from the efforts to increase the absorption and improving the pharmacokinetic profile (PK) of maraviroc (**1**). The structure of PF-232798 (**90c**) resulted from an alternative approach which intended to circumvent the CYP 2D6 and hERG activity of the HTS lead UK-107,543 (**51**). The introduction of the tropane substructure instead of the piperidine moiety was previously proven to reduce CYP inhibition [1] and was therefore incorporated into the new lead compound. The lipophilic imidazopyridine and benzimidazole substructures of **51** and **78** were shown to be responsible for the inhibition of CYP 2D6 and high hERG binding [80]. In order to prevent lipophilic interactions with the hERG ion channel, the imidazopyridine substructure was replaced by more polar 1,4,6,7-tetrahydro-imidazo[4,5-c]pyridine, which led to the 3-substituted (**89a–c**) and 1-substituted (**90a–d**) series of compounds. The methyl carbamates **89a** and **89b** demonstrated high hERG inhibition. Reducing the size of the amide substituent

Table 24 3-substituted (**89a–c**) and 1-substituted (**90a–d**) 1,4,6,7-tetrahydro-imidazo[4,5-c] pyridines



89



90

Compounds	*X*	R^1	R^2	Membrane fusion IC_{50} (nM)[a]	hERG channel inhibition[b]
89a	H	H_3CO–	H_3C–	0.6	57%
89b	F	H_3CO–	H_3C–	0.2	28%
89c	F	H_3C–	H_3CO–	0.1	0%
90a	F	H_3C–	H_3CO–	<0.1	2 μM
90b	F	H_3C–	EtO–	<0.1	5 μM
90c (PF-232798)	F	H_3C–	^{i}PrO–	<0.1	12 μM
90d	F	H_3C–	^{t}BuO–	<0.1	6 μM

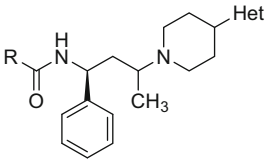
[a]Inhibitory effect on gp160 fusion
[b]Inhibition of [^{3}H]dofetilide binding to hERG stably expressed on HEK-293 cells

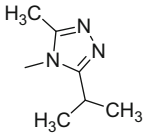
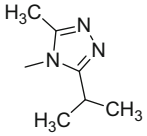
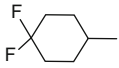
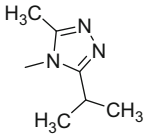
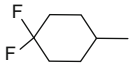
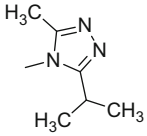
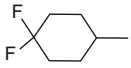
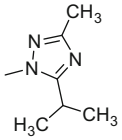
to an acetyl group (**89c**) significantly increased the selectivity for hERG ion channel within the series **89.** Also the hERG affinity was reduced by incorporation of a *m*-fluoro substituent into the phenyl ring (**89b**). Switching the substitution position of the 1,4,6,7-tetrahydro-imidazo[4,5-c]pyridine slightly improved the gp160 inhibition from **89c** to **90a**, but was detrimental in terms of hERG binding. The lowest hERG inhibition ($IC_{50} = 12$ μM) could be achieved by introduction of an isopropoxycarbonyl substituent **90c** (Table 24). Compound **90c** demonstrated complete oral absorption in rat and dog that was accompanied by improved metabolic stability compared to maraviroc (**1**) and other compounds in this series. Moreover, PF-232798 (**90c**) displayed antiviral activity against maraviroc-resistant viruses and was therefore chosen as the follow-up clinical candidate of maraviroc (**1**) [98].

3.2.3 1-Amido-1-Phenyl-3-Piperidinylbutane-Based CCR5 Ligands

The growing number of reports on maraviroc-resistant HI-viruses [93, 99, 100] underlines the need for development of a next generation of ligands with different resistance profile. The tropane-core represents the central structural motif of all previously successfully developed CCR5 ligands. The key features of the tropane moiety are an increased steric hindrance around the basic amino group and restricted conformational flexibility of the molecule. In order to retain the steric

Table 25 Heterocycle-substituted piperidines **91–94** bearing an α-methyl moiety



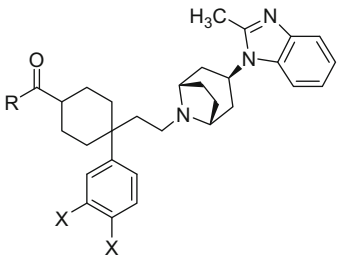
Compounds	*R*	Het	Membrane fusion IC_{50} (nM)[a]	hERG inhibition IC_{50} (μM)[b]
91			4.3	2.1
92			1.3	2.3
(*R*)-**93**			0.48	>10
(*S*)-**93**			50	>10
(*R*)-**94**			3.0	>10

[a]Inhibitory effect on gp120-sCD4 complex binding
[b]Inhibition of [^{3}H]dofetilide binding to hERG stably expressed on HEK-293 cells

hindrance, the introduction of an additional methyl moiety into the propyl chain was envisaged. Several heterocycles as well as different amido substituents were screened for their antiviral activity, oral bioavailability, and low propensity towards hERG ion channel inhibition and interaction with a range of CYP enzymes. A large series of piperidines **91**–**94**, substituted with a *N*-heterocycle, bearing an α-methyl moiety were prepared (Table 25) [101, 102].

The cyclobutyl derivate **91** reveals high antiviral activity combined with the low hERG inhibition. Introduction of two additional fluorine atoms at the alkyl substituent (**92**) was shown to be beneficial in terms of antiviral activity, as it was already observed during the optimization of maraviroc [89]. Increasing the size of the alkyl substituent to a cyclohexyl group (**93**) inhibits the interactions with the hERG

Table 26 First, potent 4,4-disubstituted piperidine-based CCR5 ligands **95** and **96**



Compounds	*R*	*X*,*Y*	CCR5 IC_{50} (nM)[a]	Antiviral activity IC_{50} (nM)[b]
95		H, H	16.8	–
96		Cl, H	1.0	16.8

[a]Inhibitory potency (IC_{50}) of the [^{125}I]-CCL4 binding to the human CCR5 receptor
[b]Inhibitory potency of replication of HIV-Bal in PM-1 cells

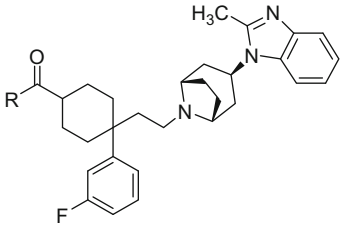
channel leading to an affinity higher than 10 μM. Enantioselective synthesis of **93** allowed the determination of (*R*)-**93** as eutomer, with a 100-fold higher antiviral activity than the (*S*)-enantiomer. Bioisosteric replacement of the 1,3,4-triazolyl residue of (*R*)-**93** by a 1,2,4-triazol-1-yl ring in (*R*)-**94** resulted in a highly metabolically stable and potent CCR5 ligand. The 1,2,4-triazole (*R*)-**94** displays excellent whole-cell antiviral activity (IC_{90} = 2.6 nM), complete oral absorption in rat, and does not inhibit human CYP 1A2, 2C9, and 2D6 enzymes. Moderate affinity towards CYP 3A4 (IC_{50} = 3.4 μM) was also found [101, 102]. Therefore, compound (*R*)-**94** represents the new lead compound for the development of novel CCR5 ligands with new resistance profile.

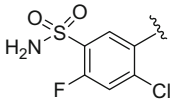
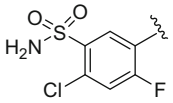
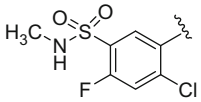
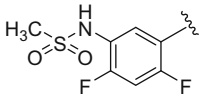
3.2.4 4,4-Disubsituted Piperidine-Based CCR5 Ligands

GlaxoSmithKline's approach combining in-house HTS with computer-assisted drug design resulted in identification of two 4,4-disubstituted piperidines **95** and **96** (Table 26). Compounds **95** and **96** show high CCR5 affinity and high antiviral activity combined with promising pharmacokinetic profile in rodents. Unfortunately, **95** and **96** showed also moderate hERG affinity of IC_{50} of 2 μM and 10 nM, respectively, which required further optimization [103].

The optimization focused on the substitution pattern of the phenyl rings, which led to the discovery of several potent CCR5 ligands with decreased inhibition of hERG. Introduction of a sulfonamide and two halogen substituents at the phenyl ring (**97**) turned out to decrease hERG affinity and retained antiviral activity. The exchange of the positions of the fluoro and chloro atoms from **97** to **98** decreased

Table 27 Potent 4,4-disubsituted piperidine-based CCR5 ligands **97–100** with decreased inhibition of hERG



Compounds	*R*	Antiviral activity IC_{50} (nM)[a]	hERG inhibition IC_{50} (μM)[b]
97		4.4	>32
98		43	>57
99 (GSK163929)		4.3	19
100		6.2	100

[a]Inhibitory potency of replication of HIV-Bal in PM-1 cells
[b]hERG patch-clamp assay

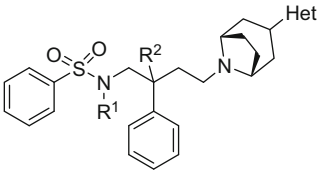
the antiviral activity. The reverse sulfonamide **100** is less hydrophobic than the forward sulfonamide **99** and was found to be very potent in anti-HIV assays (Table 27) [104]. However, compound **99** (GSK163929) was favored for further investigation as a clinical candidate, due to the potential aniline metabolite formation from reverse sulfonamide **100**.

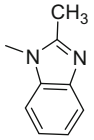
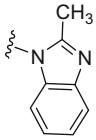
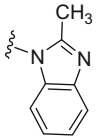
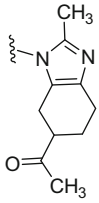
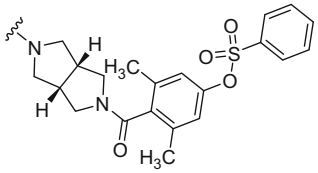
GSK163929 (**99**) revealed high oral bioavailability and good pharmacokinetic profile in rats and dogs. Seven-day safety studies in both species did not show any adverse effects at therapeutic doses. The high antiviral activity, favorable pharmacokinetic profile, and safety data support further development of **99** in phase I clinical studies [104].

3.2.5 2-Phenylbutane-1,4-Diamine-Based CCR5 Ligands

Investigations of the GlaxoSmithKline research laboratories revealed a new class of CCR5 ligands with a 2-phenylbutane-1,4-diamine core structure. The sulfonamide

Table 28 Antiviral activity of 2-phenyl-1,4-butanediamine-based CCR5 ligands **101**–**105**. Inhibitory effect on replication of HIV-Bal in PM-1 cells



Compounds	R^1	R^2	Het	Antiviral activity IC_{50} (nM)
101	H_3C–	H–		8
102	H–	H–		65
103	H_3C–	H_3C–		9
104	H–	H–		3
105	H–	H–		8

substituent turned out to produce superior antiviral activity over all other amides tested. Removal of the methyl substituent at the amide N-atom of the benzimidazole derivative **101** led to an 8-fold loss in the antiviral activity (**102**). Introduction of a second methyl moiety at position 3 of the butanediamine linker (**103**) increased the activity (Table 28). Compounds bearing other heterocycles than benzimidazole were less sensitive to the effect of *N*-methyl substitution [105].

Compounds **102** to **104** exhibit moderate to fast clearance, only **101** and **105** showed measurable bioavailability. All derivatives were rapidly metabolized and therefore further optimization is required in order to increase metabolic stability and improve the pharmacokinetic profile [105].

3.3 Conclusion

The development of TAK-779 (**15**), maraviroc (**1**), and their follow-up compounds exemplifies the SARs of CCR5 receptor ligands and the existing hurdles. The CCR5 receptor antagonist maraviroc (**1**) developed by Pfizer has already been approved for the treatment of confirmed R5-tropic HIV-1 infection [2]. However, the increasing number of maraviroc-resistant HI-virus strains makes the development of ligands with a distinct resistance profile highly desirable [93]. The role of CCR5 receptors in the development and progression of inflammatory diseases led to an increased interest of using CCR5 antagonists for the treatment of rheumatoid arthritis [106]. Unfortunately, the use of **1** for the treatment of rheumatoid arthritis failed due to low efficacy [107]. The reasons for this failure remain to be elucidated.

4 Dual CCR2/CCR5 Ligands

According to the involvement of both CCR2 and CCR5 receptors in the pathogenesis of inflammatory diseases [108], these receptors have become attractive targets for the pharmaceutical industry. The chemokine system is very complex, and the CCR2 receptor binds multiple endogenous ligands including CCL2, which binds exclusively to the CCR2 receptor, as well as CCL8, CCL7, and CCL13 [109] which are rather unselective. The CCR2 receptor is abundantly expressed on blood monocytes and regulates their migration from the bone marrow into inflamed tissue, whereas the CCR5 receptor is expressed on macrophages. The in vivo function of the CCR5 receptor is less well defined than that of the CCR2 receptor, but has been shown to be related to the activation, survival, and retention of macrophages in the core of inflammation and is associated with Th1 cell recruitment and activation. The CCR5 receptor also binds various ligands, including CCL3, CCL4, CCL5, CCL8, and CCL3L1. CCL4 and CCL3 are known to be selective for the CCR5 receptor. During the differentiation of monocytes, a reciprocal pattern of expression and function of the CCR2 and CCR5 receptor was observed, showing a downregulation of CCR2 and an upregulation of the CCR5 receptor expression [110, 111]. CCR2 and CCR5 receptors are expressed on different cells but in a complementary manner. Both receptors are important in the mediation of leukocyte trafficking in case of inflammation, e.g., during the pathogenesis of cardiovascular (atherosclerosis) and immunological diseases (rheumatoid arthritis, Crohn's

disease, transplant rejection). Thus targeting both receptors with dual antagonists appears to have therapeutic potential [112].

4.1 Benefits of Dual CCR2/CCR5 Ligands

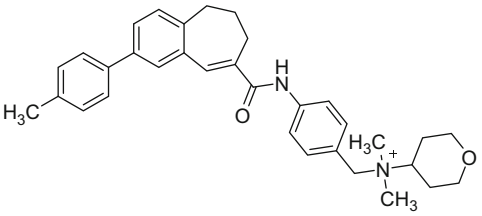
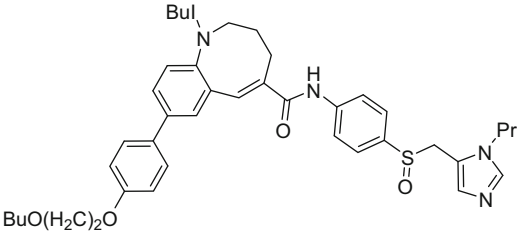
The clinical efficacy of compounds acting selectively with a particular chemokine receptor remains to be shown. Clinical trials with various chemokine ligands failed because a benefit at a critical endpoint was not shown. The complex pharmacology of chemokines and their receptors most likely contributed to the failures. More than 50 different chemokines have been identified that interact with more than 20 classical and atypical chemokine receptors. A few chemokines show a one-to-one specificity as CCL2 is specific for the CCR2 receptor, while other chemokines are promiscuous and bind to different receptors [109, 113, 114]. Also, various chemokine receptors have more than one chemokine ligand, which usually leads to differential functional response mediated by the same chemokine receptor.

Due to the fact that multiple chemokine receptors are involved in the pathophysiology of a disease, dual antagonists that target and inhibit two most prominent receptors could be a possibility to enhance the therapeutic effect. A successful example for a dual antagonism in the field of GPCRs is PS433540, which inhibits the AT_1 receptor and the ET_A receptor. PS433540 appears to be successful as antihypertensive in rats and reached phase IIa clinical trials. Therefore, promiscuous compounds that target several receptors are suggested to be particularly effective for the treatment of complex diseases, for example, multiple sclerosis or rheumatoid arthritis, in which both CCR2 and CCR5 receptors are involved [41].

4.2 Sequence Alignment of CCR2 and CCR5 Receptors

As CCR2 and CCR5 receptors belong to the same subfamily (CC) of chemokine receptors their amino acid sequences are highly homologous, mainly in the transmembrane (TM) domains [62, 115]. Both receptors contain two conserved disulfide bridges Cys32-Cys277 and Cys113-Cys190 in the CCR2 receptor and Cys20-Cys269 and Cys101-Cys178 in the CCR5 receptor. Comparative analysis revealed 66 % sequence identity in general between CCR2 and CCR5 receptors and 82 % identity in the active site. Receptor homology modeling studies predicted a ligand binding pocket of the CCR2 receptor formed by TM2, 3, 5, 6, and 7. In the CCR5 receptor TM1, 2, 3, 5, 6, and 7 form the binding pocket for CCR5 inhibitors, which is located at the extracellular region and is partly covered by the extracellular loop. Both CCR2 and CCR5 receptors possess glutamic acid in the anchor site in TM 7, Glu291 in CCR2 receptors, and Glu283 in CCR5 receptors. Glu291/Glu283 is essential for the interaction with protonated tertiary amines or quaternary ammonium ions. Superposition of CCR2/CCR5 binding sites revealed that all residues are

Table 29 Dual CCR2/CCR5 antagonists, TAK-779, and TAK-652

Compounds		CCR2 IC_{50} (nM)[a]	CCR5 IC_{50} (nM)[b]
15 (TAK-779)		27	1.4
43 (TAK-652)		5.9	3.1

[a]Inhibitory effect on binding of CCL2 to human CCR5 receptor
[b]Inhibitory effect on binding of [^{125}I]-CCL5 to human CCR5 receptor

identical except three: Ser101/Tyr89, His121/Phe109, and Arg206/Ile198, which differ considerably in their electronic and hydrophobic properties.

Contemporary modeling studies performed on the basis of the 2010 crystallized CXCR4 receptor show higher sequence homology to CCR2/CCR5 than prior used templates based on bovine rhodopsin or the β2-adrenergic receptor [28]. The recently reported X-ray crystal structure of the CCR5 receptor/maraviroc complex [95] will allow a deep insight in the binding site and sophisticated modeling studies.

4.3 TAK-779 and TAK-652

The dual CCR2/CCR5 antagonist TAK-779 (**15**) (Table 29) is the most extensively investigated compound regarding binding site experiments and computational predictions. Using computational calculations, low-energy three-dimensional receptor conformations of human CCR2 and CCR5 receptors were created, and the binding sites of **15** within the CCR2 and CCR5 receptor were predicted. Mutation experiments in which single amino acids were replaced within the receptor structure were performed, and after transient expression in the L1.2 cells chemotactic and competitive binding experiments to CCR2 and CCR5 receptors were carried out. Based on these data, it was postulated that Trp98/Thr292 in the CCR2 receptor (Fig. 3) and Trp86/Tyr108 in the CCR5 receptor (Fig. 5) were significantly associated with the

efficacy of TAK-779. His121 in the CCR2 receptor was also important for antagonistic efficacy and was replaced by Tyr108 in the CCR5 receptor. An altered rotational orientation of TM3 is responsible for a different positioning of these aromatic residues in both CCR2 and CCR5 receptors. A comparison of quaternary ammonium salt TAK-779 (**15**) with antagonists including a tertiary amine showed differences in binding of these interacting residues. Depending on Glu291 in the CCR2 receptor and Glu283 in the CCR5 receptor facing either TM1 and TM2 or TM3 and TM6, different orientations of TM7 are possible: The first receptor conformation leads to a receptor activation network formed between TM 1, 2, 3, and 7, which is supposed to be required for the receptor activation by the chemokine. The second conformation is expected to be stabilized by antagonist binding [62].

The interaction of TAK-779 (**15**) with the respective binding sites of CCR2 and CCR5 receptors resulted in IC_{50} values of 27 nM for the CCR2 receptor and 1.4 nM for the CCR5 receptor. **15** also inhibited the binding of CCL3 and CCL4 to the cells expressing the CCR5 receptor with an IC_{50} value of 1.0 nM. No interaction was found between TAK-779 and CCR1, CCR3, CCR4, or CXCR4 receptors [71].

TAK652 (**43**) (Table 29), a benzazocine compound, was developed by Takeda Inc. as a CCR5 antagonist for anti-HIV-1 therapy in order to improve the poor oral bioavailability of the quaternary ammonium salt TAK-779.

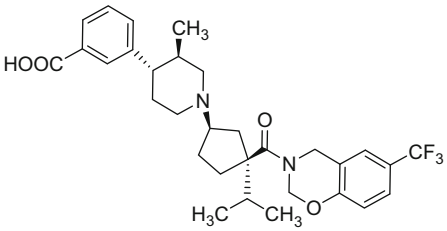
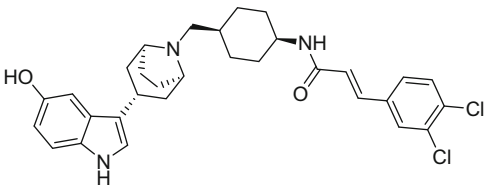
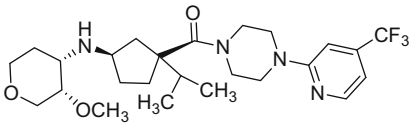
In addition to the high CCR5 affinity ($IC_{50} = 3.1$ nM in [^{125}I]-CCL5 assay), TAK-652 was also found to be a potent CCR2 antagonist with binding affinity of 5.9 nM [116]. This effect was neither observed for TAK-220 (**48**) and maraviroc (**1**) nor any other CCR5 ligand [117].

4.4 MK0483

As described in Sect. 2, compounds with an aminocyclopentanecarboxamide scaffold were developed as CCR2 antagonists with promising receptor affinity but also significant hERG inhibition. MK0483 (**6**) (Table 30) showed high CCR2 affinity ($IC_{50} = 4$ nM, measured as inhibition of [^{125}I]-CCL2 binding) and displayed an IC_{50} value of 0.3 nM in chemotactic assays. MK0483 inhibited [^{125}I]-CCL3 binding to CCR5 receptors with an IC_{50} value of 25 nM. Additionally a low ERG affinity ($IC_{50} = 33$ μM) was found. The improved lack of hERG inhibition with regard to Merck's previous compounds was shown to be associated with the 3-carboxyphenyl in position 4 of the piperidine of **6** [118].

A broad screening against different receptors, CYP enzymes and ion channels displayed high selectivity for CCR2 and CCR5 and weak interaction with muscarinic receptors M_2 and M_4 without any interaction with CYP enzymes, including CYP 3A4, 2C9, 2D6, 1A2, and 2C19. Efficacy of **6** was also evaluated in different rhesus blood experiments, suggesting that subnanomolar potency can be achieved in vivo [118].

Table 30 Dual CCR2/CCR5 antagonists MK0483, SKB3380732, and INCB10820/PF-4178903

Compounds		CCR2 IC_{50} (nM)[a]	CCR5 IC_{50} (nM)[b]
6 (MK0483)		4	25
106 (SKB3380732)		40	4,000
107 (INCB10820/PF-4178903)		3	5.3

[a]Inhibitory effect on binding of CCL2 to human CCR2 receptor
[b]Inhibitory effect on binding of [^{125}I]-CCL5 to human CCR5 receptor

4.5 SKB3380732

The indolyltropane SKB3380732 (**106**) (Table 30), developed as a potent CCR2 ligand, displayed an IC_{50} value of 40 nM for the CCR2 receptor. The development of **106** started from a potent CCR2 antagonist with an indolylpiperidine scaffold. It showed selectivity over the CCR5 receptor, but due to its high structural similarity with serotonin (5-hydroxytryptamine), this indolylpiperidine was not selective and interacted with several serotonergic and dopaminergic receptors. In order to improve the selectivity, a conformational restriction of the indolylpiperidine via a tropane moiety was performed, and the steric bulk around the basic amine was increased. Moreover, the flexible pentyl chain was exchanged by a methylcyclohexyl linker. The conformational constraint of both the piperidine ring and the pentyl alkyl chain led to 1,000-fold increased CCR2 selectivity over a number of serotonin and dopamine receptors but retained high CCR2 affinity. In contrast to previous compounds, **106** showed moderate CCR5 affinity (IC_{50} = 4,000 nM) (Table 30) [41, 119].

4.6 INCB10820/PF4178903

Incyte and Pfizer discovered a series of dual CCR2/CCR5 antagonists, leading to INCB10820/PF-4178903 (**107**) (Table 30) as the most potent compound. SAR studies on both the left and right part of the molecule, containing an aminocyclopentanecarboxamide with an isopropyl moiety, resulted in **107** as most promising dual antagonist.

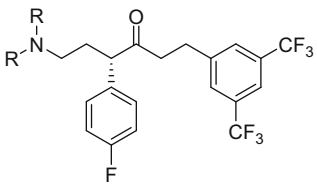
Compound **107** displayed a CCR2 affinity of $IC_{50} = 3.0$ nM in [^{125}I]-CCL2 assay and $IC_{50} = 5.3$ nM to the CCR5 receptor in [^{125}I]-CCL4 binding assay. Compared the analog bearing a 3-trifluoromethylphenyl instead of the 3-trifluoromethylpyridin-2-yl moiety (**107**), the CCR5 affinity increased 4-fold. Regarding to chemotactic activity, **103** showed similar IC_{50} values in both CCR2 ($IC_{50} = 3.2$) and CCR5 ($IC_{50} = 4.3$ nM) binding assays. A further replacement of the 3-trifluoromethylpyridin-2-yl moiety by the 4-trifluoromethylpyrimidin-2-yl residue provided a less active analog. Screening of the affinity towards various receptors, enzymes, and ion channels (>50) indicated **107** to be a selective and dual CCR2 and CCR5 antagonist. **107** did not inhibit CYP 3A4 and 2D6 enzymes, but inhibited the hERG channel ($IC_{50} = 1.7$ μM). Due to the promising in vivo properties with oral bioavailability of 84% in rats and 57% in monkeys and high metabolic stability ($t_{1/2} = 93$ min), **107** became a candidate for clinical studies [120].

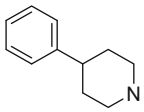
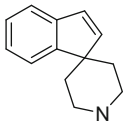
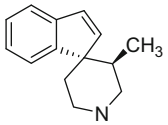
The binding of **107** was also analyzed by docking into the binding site of the CCR2 and CCR5 receptor. The tertiary amine of **107** forms a salt bridge with the acidic residues Glu291 (CCR2) and Glu283 (CCR5). The trifluoromethyl substituent was also identified to interact with Arg206 of the CCR2 receptor and Ile 198 of the CCR5 receptor [28].

4.7 γ-Aminobutyramides

As detailed in Sect. 2, several CCR2 antagonists from various chemical classes have already been reported. Merck developed further a new class of promising CCR2 antagonists based on the γ-aminobutyramide core. The screening of Merck's sample collection led to some lead compounds, which upon further optimization resulted in ligands **108**, **109**, and **110** (Table 31) with both CCR2 and CCR5 antagonistic properties. A high structural similarity to known CCR5 antagonists was achieved by incorporation of a substituted piperidine ring. Compound **108** with the 4-phenylpiperidine moiety demonstrated moderate binding affinity to the CCR2 receptor ($IC_{50} = 150$ nM) as well as to the CCR5 receptor ($IC_{50} = 72$ nM). **108** showed improved potency compared to previously characterized unsubstituted piperidine analogs. Further variations of the phenylpiperidine moiety by 3-phenylazetidine, 3-phenylpyrrolidine, and 4-phenylazepane showed that the piperidine ring was best in terms of CCR2 and CCR5 affinity. The spiro[indene-piperidine] **109** was about 2-fold more active with IC_{50} values of 80 nM (the CCR2

Table 31 Chemical structure of dual CCR2/CCR5 antagonists with the γ-aminobutyramide substructure



Compounds	NR_2	CCR2 IC_{50} (nM)[a]	CCR5 IC_{50} (nM)[b]
108		150	72
109		80	30
110		59	26% at 10

[a]Inhibitory effect on binding of CCL2 to human CCR5 receptor
[b]Inhibitory effect on binding of [^{125}I]-CCL5 to human CCR5 receptor

receptor binding affinity) and 30 nM (the CCR5 receptor binding affinity), whereas its closely related saturated spiro[indane-piperidine] analog was less potent. The introduction of a methyl group in various positions of the γ-aminobutyramide backbone and the piperidine ring decreased potency with the exception of a methyl moiety in the 3-position of the piperidine ring. However, the CCR2 and CCR5 affinity is strongly dependent on the relative and absolute configuration of the piperidine derivatives.

Compound **110** with (*R,R,S*)-configuration was the most potent ligand from this series with the IC_{50} value of 59 nM in the CCR2 binding and an 26% inhibition at 10 nM in CCR5 binding. In chemotactic assays, progression from **108** to **109** and **110** was observed: **108** inhibited the CCL2 stimulated monocyte chemotaxis by only 40% at a concentration of 1 μM, while **109** with an IC_{50} value of 176 nM and **110** with an IC_{50} value of 41 nM. In selectivity screenings, compounds **108**, **109**, and **110** were found to be highly selective against CCR1, CCR3, CXCR3, CCR4, CXCR4, and CCR8 receptors. Only the pharmacokinetic properties of **109** were evaluated in a rat model, which showed good pharmacokinetic parameters and proper oral bioavailability at 3 mg/kg body weight [121].

5 Conclusions

The emerging evidence for the role of CCR2 and CCR5 receptors in human inflammatory diseases led to a growing interest in selective and dual CCR2/CCR5 antagonists. The availability of potent CCR2 and CCR5 antagonists allows the selective targeting of these receptors and the development of novel concepts for the therapy of inflammatory diseases (e.g., multiple sclerosis and atherosclerosis).

Maraviroc (**1**) is up to now the only commercially available CCR5 antagonist for the treatment of HIV-1 infections, but the growing number of reports on maraviroc-resistant viruses underlines the need of new drugs with improved resistance profile. During the development of new lead compounds, many issues like hERG channel affinity and metabolic stability had to be considered. The most promising CCR5 antagonists for the treatment of HIV infections are GSK163929 (**99**) and PF-232798 (**90c**), which will enter clinical studies.

The development of clinical candidates targeting the CCR2 receptor is also associated with the optimization of several aspects. Although the potent CCR2 antagonist MK0812 (**5**) has reached phase II clinical trials, the further development was terminated due to no significant improvement compared with placebo. The CCR2 antagonist JNJ17166864 (**16**) was tested in clinical trials for the local treatment of allergic rhinitis. However, the study was terminated due to lack of efficacy. In contrast to promising results in preclinical animal models of inflammation, CCR2 antagonists do not show sufficient efficacy in clinical trials of inflammatory diseases so far.

The clinical trials performed with selective CCR2 and CCR5 antagonists suggest that targeting a single receptor might not be sufficient for high efficacy. The fact that both receptors are important in the pathogenesis of cardiovascular and/or immunological diseases indicates great therapeutic potential of dual antagonists. Many compounds that were originally developed as selective antagonists of CCR2 or CCR5 receptors have shown later to address both subtypes. The systematic development of dual CCR2/CCR5 antagonists resulted in INCB10820 (**107**) as the most promising antagonist. In addition to the important central amine, **107** contains a fluoro substituent which interacts with Arg206 of the CCR2 receptor and Ile198 of the CCR5 receptor.

The recently published X-ray crystal structures of the CXCR4 and CCR5 receptors represent the basis for docking studies and virtual screening campaigns, which might lead to discovery of innovative ligands and the generation of novel selective and dual antagonists with desired pharmacological properties.

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