Recent Advances in the Development of Small-molecule CCR5 Inhibitors for HIV

Tao Liu*, Zhiyong Weng, Xiaowu Dong and Yongzhou Hu

College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China

Abstract: CCR5 (C-C chemokine receptor type 5) is a chemokine receptor that has been identified as a major HIV co-receptor in viral entry and therefore is a highly validated target for the development of new anti-HIV drugs. Here, we discuss the insights gained so far relevant to the development of small-molecule CCR5 inhibitors for the treatment of HIV, and highlight small-molecule CCR5 inhibitors that are currently under preclinical and clinical trials.

Keyword: HIV, Entry, Co-receptor, CCR5, Small-molecule inhibitor, Maraviroc.

1. INTRODUCTION

Acquired immune deficiency syndrome (AIDS), mainly caused by human immunodeficiency virus type 1 (HIV-1), remains among the leading causes of death globally. According to a report on the global AIDS epidemic 2008 from WHO, an estimated 33 million people worldwide were living with AIDS, with 3 million new cases and 2 million people died from AIDS in 2007 [1], bringing the cumulative number of deaths to 24 million since 1980 [2].

Current therapeutic intervention in HIV-1 infection relies upon 25 different drugs which have been formally approved for clinical use [3]. Treatment with antiretroviral agents, especially with highly active antiretroviral therapy (HAART) regimens using cocktails of reverse transcriptase and protease inhibitors, has substantially reduced morbidity and mortality significantly, [4]. However, HAART regimens cannot eradicate HIV-1 from infected people [5], and the application of HAART regimens are limited by long-term toxicity of drugs and rapid emergence of drug-resistant HIV-1 strains [6]. Therefore, novel agents targeting different steps of the viral replication cycle, such as viral entry, are needed to overcome these problems.

HIV-1 entry is a complex process that involves enveloping glycoproteins (gp120 and gp41) on the surface of the virions, a CD4 receptor (the primary HIV-1 receptor at the cell surface), and co-receptor (the main co-receptors used by HIV-1 are chemokine receptors CCR5 and CXCR4) on the host cells, all of which are potential targets for antiretroviral intervention [7]. The entry process is initiated by gp120 binding with CD4, thereby anchoring the virus to the surface of the host cell. CD4 binding induces conformational changes in gp120, enabling additional interactions with co-receptor, such as CCR5, then inducing further conformational changes in gp41, resulting in the fusion between the viral and cellular membranes (Fig. **1**) [8].

*Address correspondence to this author at the ZJU-ENS Joint Laboratory of Medicinal Chemistry, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China; Tel: 086-571-88208458;
Fax: 086-571-88208458; E-mail: lt601@zju.edu.cn

CCR5 and CXCR4 belong to the G protein-coupled receptors (GPCR) superfamily, characterized by seventrans-membrane (TM) domains [9]. Although macrophagetropic (R5-) HIV-1 strains and T cell line-tropic (X4-) HIV-1 preferentially utilize CCR5 and CXCR4 as co-receptor respectively, some viral strains use both co-receptors (dual tropic) [10-12]. CCR5 appears to be important because macrophagetropic (R5-) HIV-1 strains predominate during the early stages of the infection [13]. And it is considered as an important target in the pathogenesis of AIDS. *In vitro*, cells cannot become infected with macrophagetropic (R5-) HIV-1 strains if they do not express CCR5 [14]. Furthermore, CCR5Δ32 homozygous individuals are ultimately resistant to R5-tropic HIV-1 infection, heterozygotes do become infected, but appear to have delayed disease progression [15, 16]. These observations suggest that CCR5 is a highly validated target for the treatment of HIV-1 infection.

The CCR5 inhibitors can be divided into two classes, large-molecule inhibitors and small-molecule inhibitors. There are several types of large-molecule inhibitors, such as natural ligands of CCR5 (Regulated upon activation normal T cell expressed and secreted (RANTES), macrophage inflammatory protein-1α (MIP-1α), and macrophage inflammatory protein-1β (MIP-1β)) [17], several amino-terminus modified derivatives of RANTES (AOP-RANTES, NNY-RANTES, PSC-RANTES) [18-20], and monoclonal antibody (PRO-140) [21]. Due to low oral bioavailability, unfavorable pharmacokinetics, and relatively high cost of these large-molecule CCR5 inhibitors, increasing attention is being attached to the discovery of small-molecule CCR5 inhibitors in recent years. Several classes of small-molecule CCR5 inhibitors, which are well documented in the several relating reviews have been identified [18, 22-31]. But not all types of the small-molecule CCR5 inhibitors are included and most of the reviews discussed small-molecule inhibitors reported before 2008. Herein, this review mainly focuses on the recent development (2007-2010) of small-molecule CCR5 inhibitors and highlights inhibitors which are currently under preclinical studies and clinical trials for HIV-1 (Table **1**).

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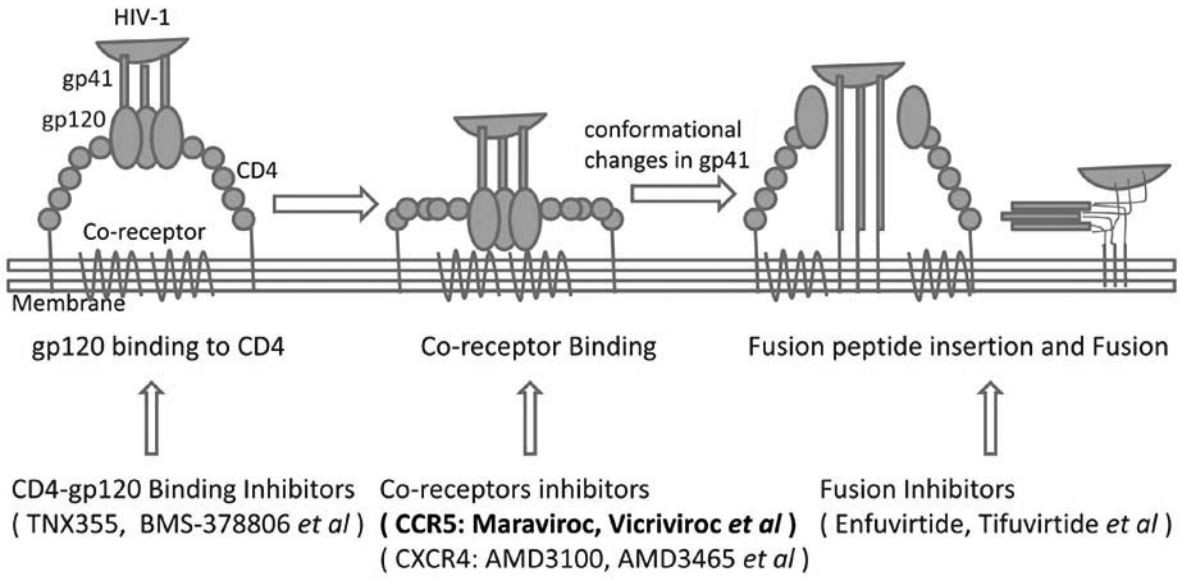


Fig. (1). HIV-1 cell entry process and potential targets for antiretroviral intervention.

Table 1. Small-Molecule CCR5 Inhibitors in Preclinical and Clinical Development

Compound	Developing company	Type of compound	Clinical phase	Status	Reference
TAK-779	Takeda	Benzocycloheptene derivatives	Preclinical	Discontinued	[39]
TAK-652	Takeda	Benzocycloheptene derivatives	Phase II	Ongoing	[43]
Aplaviroc	GSK/Ono	Spiropiperidine derivatives	Phase III	Discontinued	[48]
Ancriviroc	Schering	Piperidino-piperidine derivatives	Phase I	Discontinued	[58]
Vicriviroc	Schering	Piperidino-piperazine derivatives	Phase III	Ongoing	[60]
INCB-9471	Incyte	Piperidino-piperazine derivatives	Phase II	Discontinued	[69]
INCB-15050	Incyte	No report	Phase I	Discontinued	[68]
TAK-220	Takeda	1,4-disubstituted Piperidine derivatives	Phase I	Ongoing	[75]
Maraviroc	Pfizer	Tropane derivatives	Registered	Drug	[87]
PF-232798	Pfizer	Tropane derivatives	Phase II	Ongoing	[91]
CMPD-167	Merck	Pyrrolidine derivatives and analogues	Preclinical	Ongoing	[97]
Nifeviroc	TargetDrug/Avexa	Pyrrolidine derivatives	Phase I	Ongoing	[99, 100]

In general, except small molecules derived from natural products, smal-molecule CCR5 inhibitors can be divided into seven classes based on different structural features.

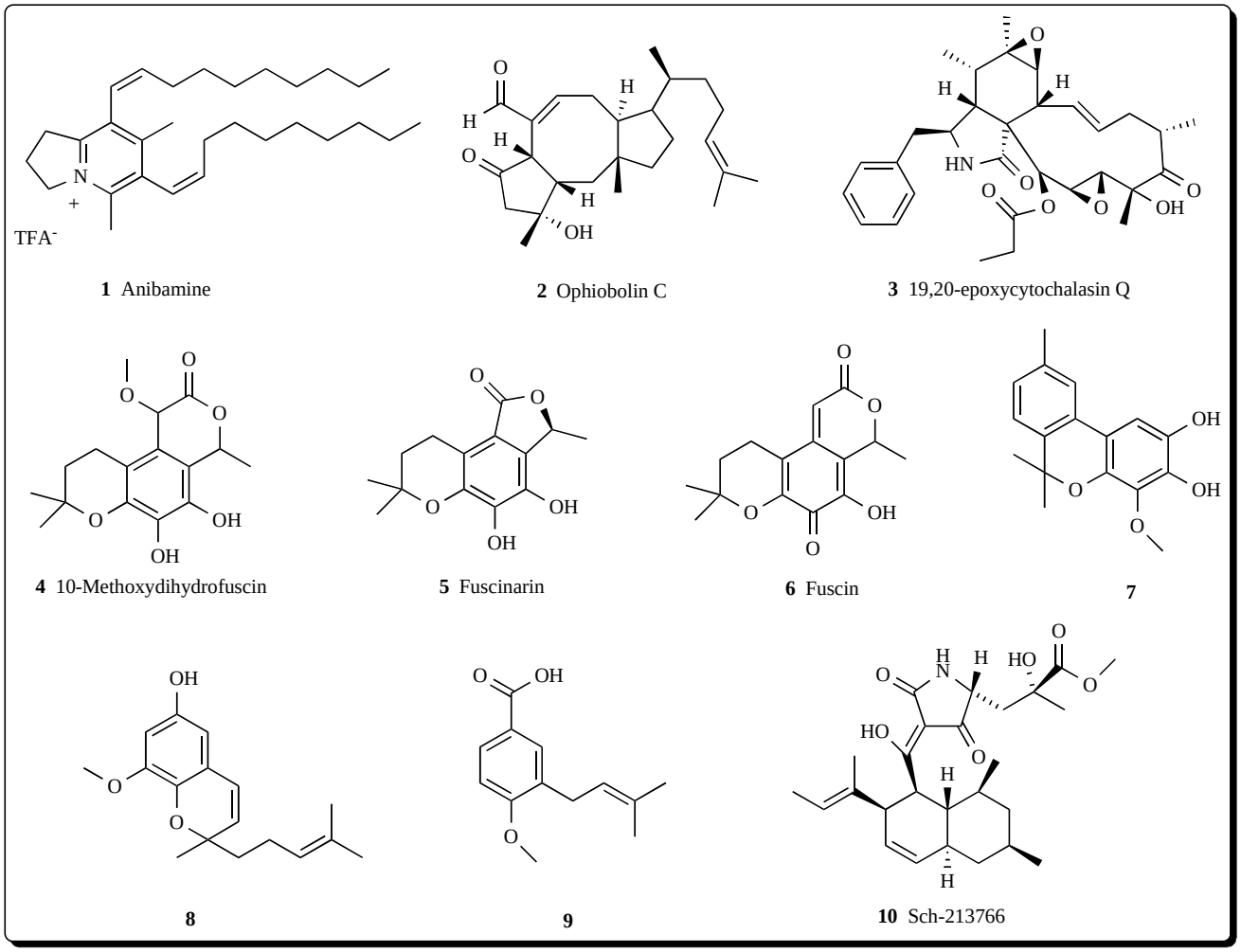
2. SMALL-MOLECULE CCR5 INHIBITORS

2.1. Natural Products

Jayasuriya and co-workers identified anibamine **1**, a novel pyridine quaternary alkaloid as a TFA salt from *Aniba* sp., discovered through screening extracts of natural products. Anibamine binds to CCR5 with an IC_{50} value of 1 μM in competition with ^{125}I-gp120. Ophiobolin C **2** from fermentation extracts of fungi *Mollisia* sp and 19,20-epoxycytochalasin Q **3** from *Xylaria* sp exhibit binding IC_{50} values of 40 and 60 μM, respectively [32].

Anibamine provides a novel structural skeleton that is remarkably different from previously identified ones. Li and co-workers reported the total synthetic routes of **1** from acetylacetone and dimethyl acetal in 11 steps with 5.1% overall yield [33]. The exploration on the synthetic routes offer the opportunity to design and prepare anibamine analogs for further biological evaluation [34].

Pentaketide metabolites, 10-methoxydihydrofuscin **4**, fuscinarin **5**, and fuscin **6**, isolated from the fungus *Oidiodendron griseum* (a mitosporic fungus), compete effectively with MIP-1α for binding to human CCR5 with IC_{50} values of 154, 80, and 21 μM, respectively [35]. Du and co-workers reported the total synthesis of **5** starting from easily available gallacetophenone in 9 steps with 33.4% overall



yield [36]. The versatility of this method was demonstrated by the synthesis of **6**.

Three compounds, 2,3-dihydroxy-4-methoxy-6,6,9-trimethyl-6*H*-dibenzo[b,d]pyran **7**, 8-methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2*H*-1-benzopyran-6-ol **8**, and 4-methoxy-3-(3-methyl-2-butenyl)-benzoic acid **9**, isolated from *Wigandia urens*, showed activity in competing with MIP-1α for binding to human CCR5 with IC_{50} values being 33, 46, and 26 μM, respectively [37]. A novel fungal secondary metabolite, Sch-213766 **10**, isolated from the fungal fermentation broth of *Chaetomium globosum*, exhibits an IC_{50} value of 8.6 μM in the CCR5 *in vitro* assay [38].

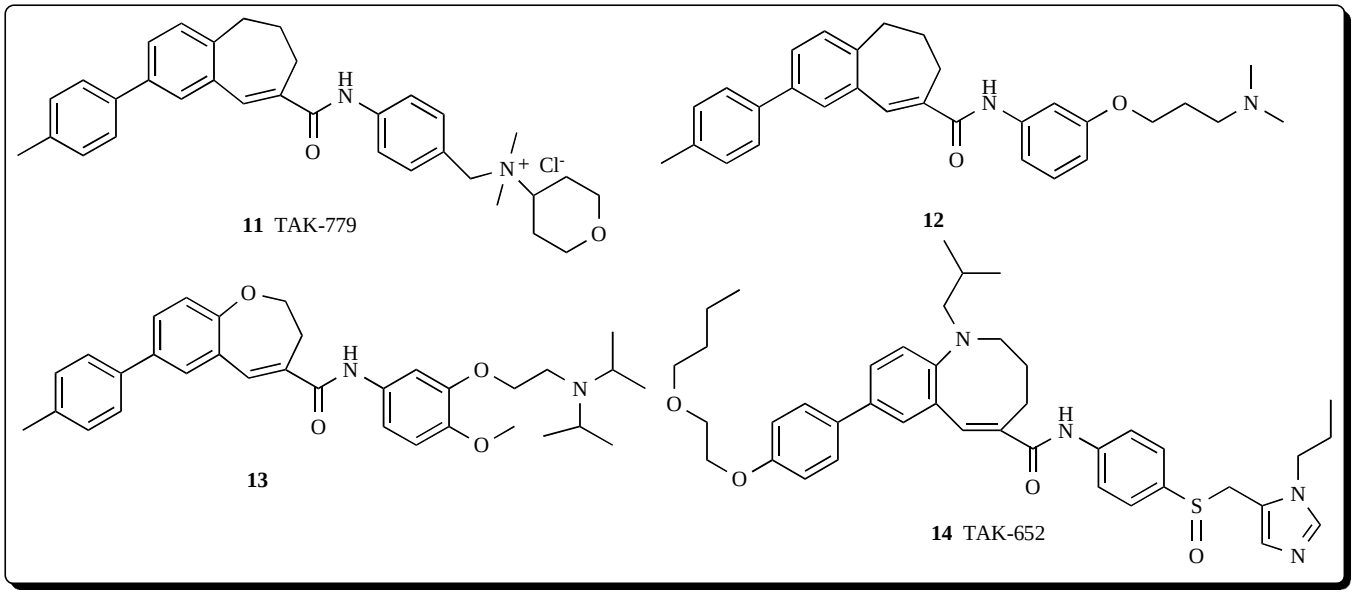
2.2. Benzocycloheptene Derivatives

As the first small molecular CCR5 inhibitor in history, TAK-779 **11** was developed through high-throughput screening (HTS) by Takeda [39]. **11** is a potent and selective nonpeptide CCR5 inhibitor with an IC_{50} value of 1.4 nM in the RANTES binding assay. It inhibits the replication of macrophage (M)-tropic HIV-1 (Bal strain) in both MAGI-CCR5 cells and peripheral blood mononuclear cells (PBMCs) with EC_{50} values of 1.2 and 3.7 nM, respectively.

To explore the interaction mechanism between **11** and CCR5, Dragic and co-workers modeled the 3D-structure of CCR5 using that of rhodopsin as a template [40]. The mutagenesis and modeling data strongly suggested that compound **11** occupies a pocket surrounded by TM helices 1, 2, 3, and 7. The depth of the pocket is approximately the same length of the methylphenyl-benzocycloheptenyl group of **11**, and this structure has distinctive hydrophobic and polar ends, so it is envisioned that the methylphenylbenzocycloheptenyl group inserts into the hydrophobic TM pocket, allowing the charged moiety to protrude and presumably interact with the polar extracellular domain.

Based on the interaction mode between **11** and CCR5, Liu and co-workers designed and synthesized a series of compounds incorporating some of the favorable pharmacophoric elements concluded recently. Anti-HIV activity assay indicated that compound **12** and **13** show potent activity with IC_{50} values of 57 and 68 nM, respectively (in TZM-bl cells infected by HIV-1 Bal(R5)) [41, 42].

Although TAK-779 showed no cytotoxicity to the host cells, the development of it was terminated for its poor oral bioavailability and the emergence of irritation at the injection site. Replacement of the quaternary ammonium moiety of **11** with a polar sulfoxide moiety, a ring expansion of (6,7)-fused nuclei to (6,8)-fused nuclei, and substitution of a 4-(2-butoxyethoxy) group for methyl group led to TAK-652 **14**



with an increase in bioavailability and potency [43]. **14** shows potent anti-HIV-1 activity (IC_{90} = 0.81 nM in MOLT4/CCR5 cells), inhibits the replication of six macrophage-tropic (CCR5-using or R5) HIV-1 clinical isolates in PBMCs (mean IC_{90} = 0.25 nM), and is well absorbed after oral administration in rats, dogs, and monkeys [44]. TAK-652, which was named TBR-652 when Takeda granted it to Tobira for further developing in Aug. 2007, is currently undergoing Phase II clinical trials.

2.3. Spiropiperidine Derivatives

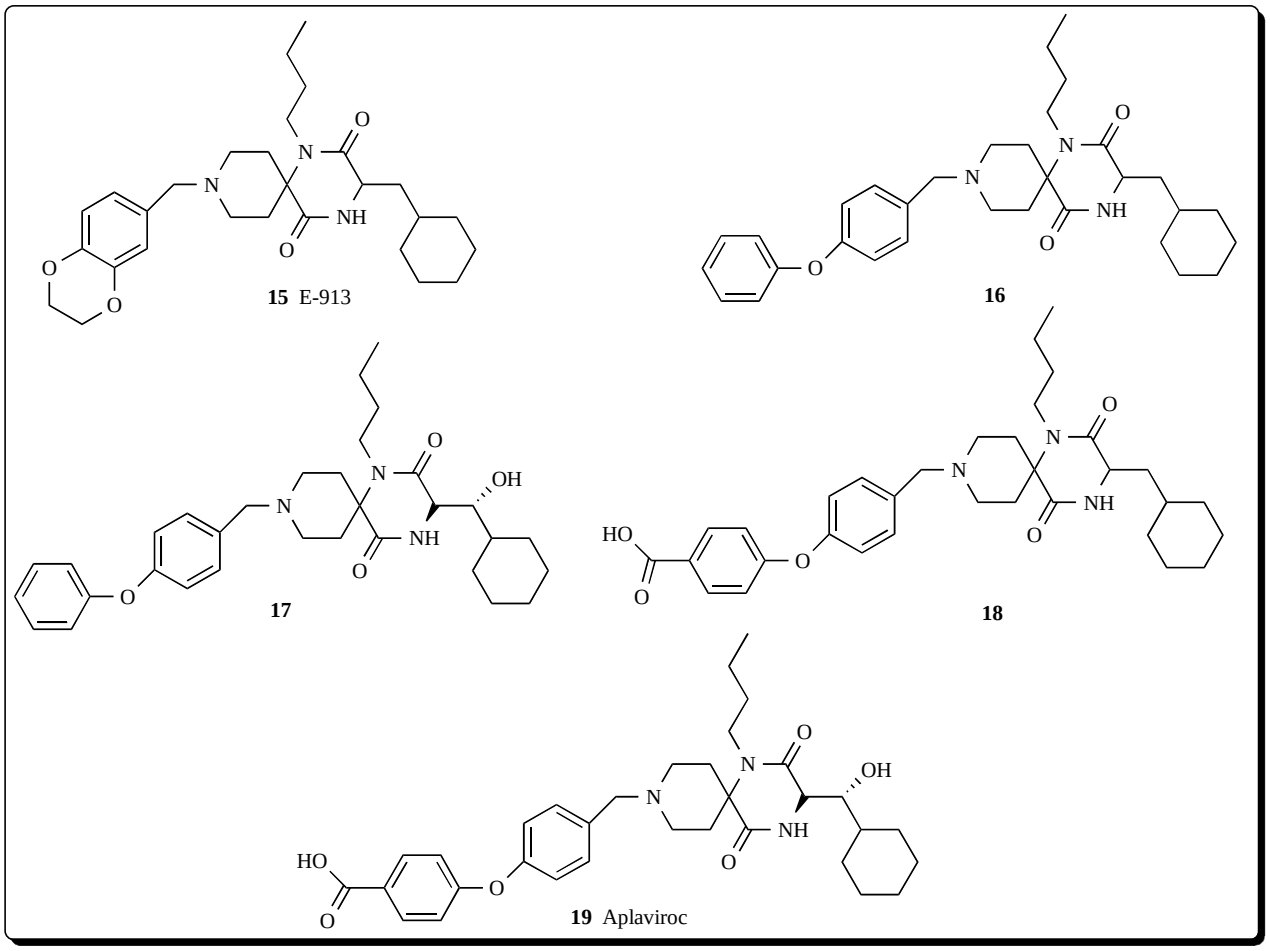
Ono Pharmaceutical Company described a new class of spiropiperidine-based CCR5 inhibitors. The lead compound E-913 **15** specifically blocks the binding of MIP-1α to CCR5 (IC_{50} = 2 nM) and MIP-1α-elicited cellular Ca^{2+} mobilization (IC_{50} = 20 nM) [45]. **15** potently inhibits the replication of laboratory and primary R5 HIV-1 strains, as well as various multidrug-resistant monocyte/macrophage tropic (R5) HIV-1 with IC_{50} values ranging from 0.03 to 0.06 μM. **15** and its analogs are acid-resistant and orally bioavailable in rodents. These data warranted that spiropiperidine derivatives will be further developed as potential therapeutics for HIV-1 infection.

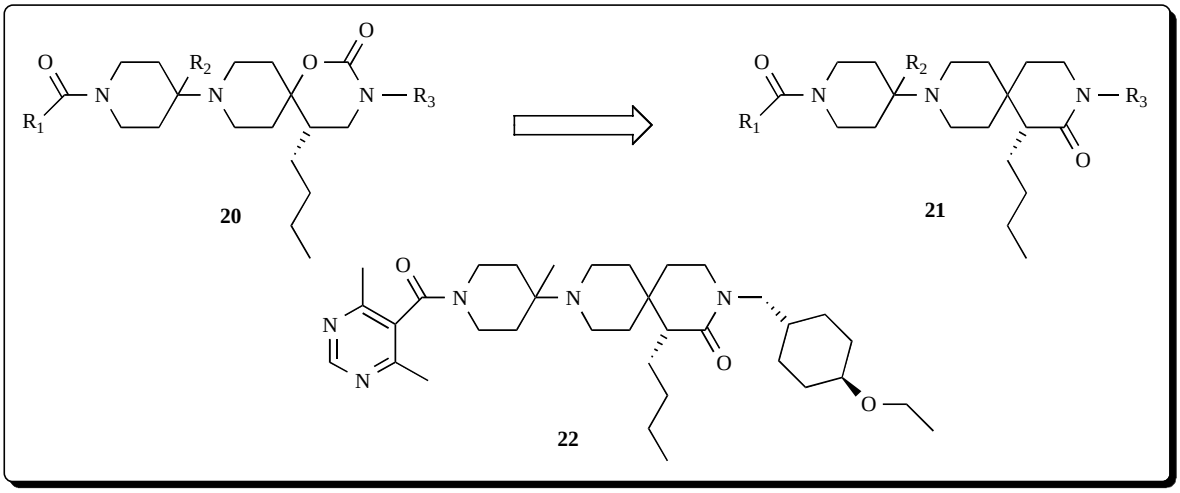
They also described the identification of several spiropiperidine derivatives, for example, compound **16**, using a combinatorial chemistry approach [46]. Although **16** shows potent activity *in vitro* (binding assay IC_{50} = 6.1 nM, anti-HIV activity IC_{50} = 31 nM), its pharmacokinetic profile is poor. After the incubation with human liver microsomes, metabolites of **16** were purified by HPLC, prompting them to introduce hydroxyl group on side chains to improve the *in vitro* activity as well as pharmaceutical properties. Compound **17** derived from β-substituted (2R,3R)-2-amino-3-hydroxypropionic acid shows improved inhibitory activities against the binding of MIP-1α to human CCR5 (IC_{50} =: 3.5 nM) compared with the non-hydroxylated derivatives and the other isomers, but the oral bioavailability of **17** in rat is less than 1%. Introduction of a *p*-carboxylic acid group as an electron-withdrawing and hydrophilic substituent in compound **16** afforded compound **18** with increased activity [47]. However, stability of it in rat liver microsomes does not show significant improvement.

Further development resulted in aplaviroc (ONO-4128, GW-873140) **19** [48], which specifically blocks the binding of MIP-1α to CCR5 with high affinity (K_d = 3 nM), potently blocks HIV-1 gp120/CCR5 binding and exerts potent activity against a wide spectrum of laboratory and primary R5 HIV-1 isolates, such as multidrug-resistant HIV-1 (IC_{50} values of 0.1 to 0.6 nM) *in vitro*. It demonstrated a 100-fold increase in activity against HIV-1 isolates over **15**. Pharmacokinetic studies revealed favorable oral bioavailability in rodents.

GlaxoSmithKline and Ono Pharmaceutical Company entered into a collaborative agreement for the clinical development of aplaviroc. It was shown that this compound exhibits rapid, extensive, specific, and prolonging binding to CCR5 in blood samples from clinical study volunteers [49], and it is generally well tolerated during short-term administration in studies of healthy volunteers and HIV-1 infected individuals [50]. Unfortunately, compound **19** was terminated in Phase III clinical trials because of apparent idiosyncratic hepatotoxicity [51]. The mechanism of the idiosyncratic hepatotoxicity of compound **19** has not been articulated clearly yet, but was believed to be intrinsic rather than connected to its novel mechanism of action [52]. Idiosyncratic hepatotoxicity is dose-related toxicity, therefore, Latinovic and co-workers demonstrated that reduction of CCR5 density (receptors/cell) with the immunomodulatory drug rapamycin enhances the antiviral activity of **19** at lower, non-toxic effective doses [53].

Efforts at Roche led to the discovery of a series of spiropiperidine derivatives as CCR5 inhibitors represented by structure **20**, featuring an 1-oxa-3,9-diazaspiro[5.5]undecan-2-one template [54, 55]. Replacement of the cyclic carbamate led to the discovery of 3,9-diazaspiro[5.5]undecane derivatives **21**. One representative compound **22** was found to have attractive antiviral potency (inhibits RANTES bind-





ing to CCR5 with an IC_{50} value of 24 nM), selectivity (shows no significant inhibition for CCR1, CCR2, CCR3, CCR4, CCR6, and CXCR4), and pharmacokinetic profile (shows no significant cytochrome P450 inhibition against major subtypes, such as 3A4, 1A2, 2C9, 2C19, and 3D6, and no significant *h*ERG inhibition activity with an *in vitro* IC_{20} value of 3 μM) [56, 57]. What described above demonstrated that 3,9-diazaspiro[5.5]undeca-2-one series could be futher developed.

2.4. Piperidino-Piperidine and Piperidino-Piperazine Derivatives

Schering developed a series of piperidino-piperidine and piperidino-piperazine derivatives, of which ancriviroc (SCH-C, SCH-351125) **23** has been in Phase I clinical trials [58]. **23** has broad and potent antiviral activity *in vitro* against primary HIV-1 isolates, with mean 50% inhibitory concentrations ranging between 0.4 and 9 nM, and has a favorable pharmacokinetic profile in rodents, which primated with an

oral bioavailability of 50-60% and a serum half-life of 5-6 h. Considering the potent antiviral activity and favorable *in vivo* pharmacokinetic profile, **23** was believed to be a promising candidate for therapeutic intervention of HIV-1 infection. Unfortunately, clinical trials of **23** demonstrated unacceptable cardiovascular and central nervous system (CNS) toxicity, presumably due to drug affinity with other receptors [59]. Thus, a secondary screen was performed to assess the anti-viral activity and cardiovascular and central nervous system (CNS) side effects of selected compounds, leading to the discovery of AD-101 (SCH-350581) **24** and AD-114 (SCH-350634) **25** [28]. Further modification on substitution pattern produced vicriviroc (SCH-D, SCH-417690) **26**, which is currently undergoing Phase III clinical trials [60].

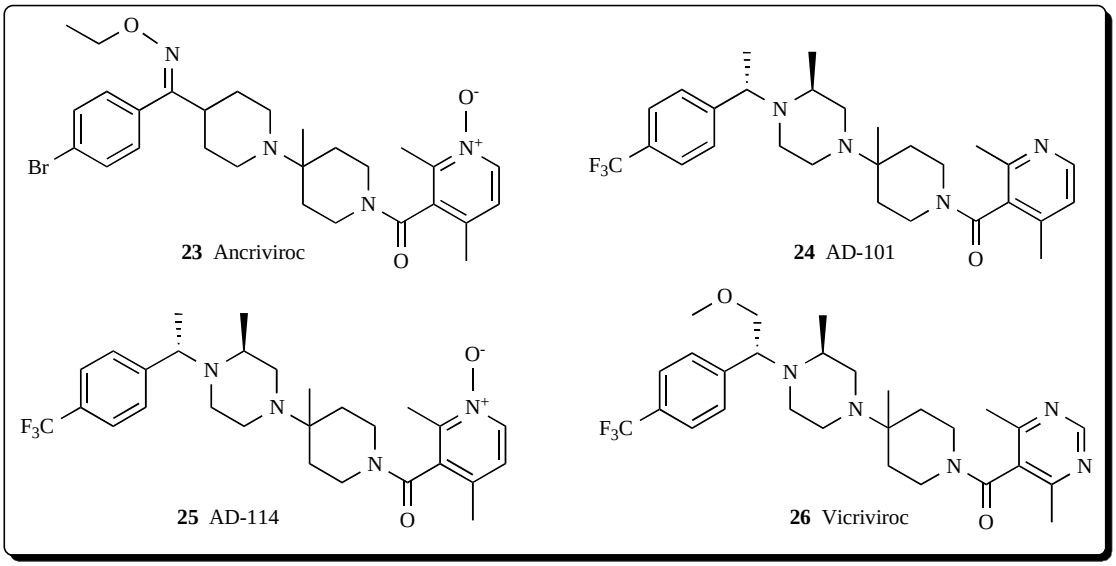
Vicriviroc **26** has high potency against a range of HIV-1 isolates (IC_{90} < 10 nM) in PBMCs, high selectivity for the CCR5 receptor (*K*i = 2.5 nM *vs.* > 10,000 nM for muscarinic M2 receptors). It not only inhibits HIV-1 entry in U-80 cells with an IC_{50} of 0.46 nM [61], but also is highly active against resistant HIV-1, including enfuvirtide-, protease inhibitor-, reverse transcriptase inhibitor- and multidrug-resistant strains, with EC_{50} values ranging from 8.7 to 32.9 nM. *In vitro* studies of co-incubation with human liver microsomes (HLM) demonstrated that **26** does not significantly inhibit the activities of cytochrome P450 1A2, 2A6, 2D6, 2C9, 3A4, or 2C19 at concentrations up to 26.7 μg/mL [62]. Compound **26** is highly orally bioavailable, shows approximately 84% of protein bound in human plasma, and exhibits a half-life exceeding 24 h supporting once daily dosing [63]. Besides, no meal restriction is needed and no HIV subtype limitation is observed [64]. Results from most clinical trials suggested that combination-administration of **26** with optimized background therapy (OBT) results in virological and immunological responses in treatment-experienced adults with CCR5-tropic virus [61]. Heredia and co-workers showed that low doses of rapamycin enhances vicriviroc antiviral activity, reduces potential toxicity, and controls emerging vicriviroc-resistant variants as well [65].

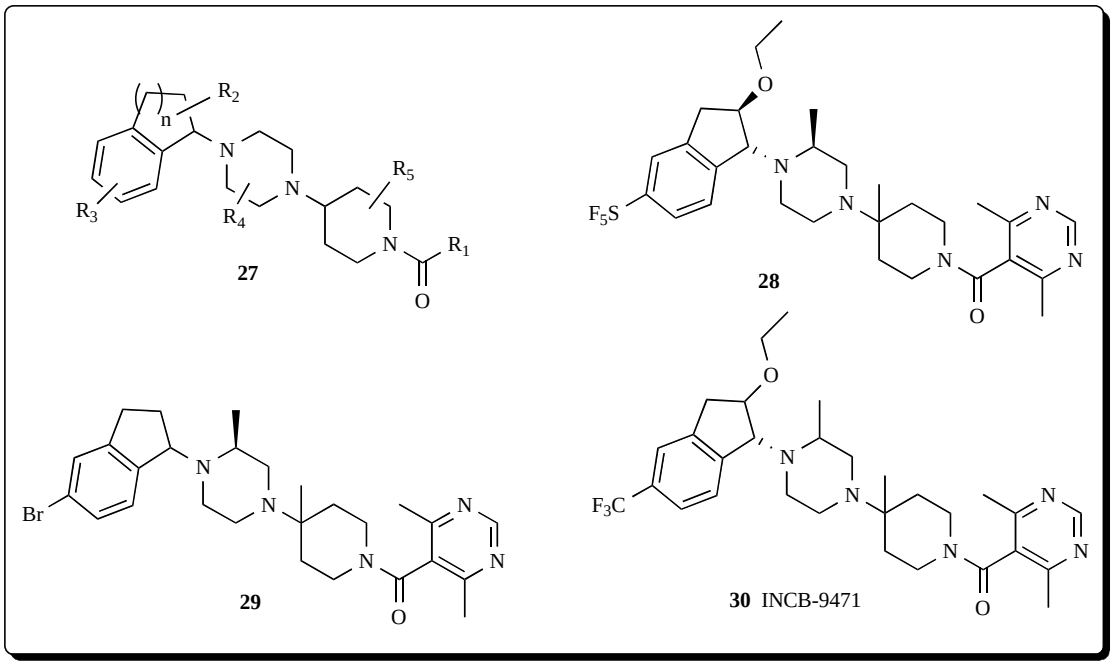
The generic structure **27** of novel series was disclosed in patents by researchers in Incyte, representative compound **28** and **29** were reported to block the binding of MIP-1β to CCR5 (IC_{50} about 1μM or less) [66, 67]. They also reported that INCB-9471 **30** and INCB-15050 (the structure has not been reported) had entered Phase II and Phase I clinical trials, respectively. But for strategic reasons, Phase II clinical trial of INCB-15050 was discontinued in order to concentrate on the development of **30** [68]. **30** potently inhibits the infection of PBMC by a wide range of R5-HIV-1 strains with an IC_{90} of 9 nM [69]. It demonstrates selectivity against other chemokine receptors, ion channels, enzymes, and transporters. Satisfactory oral pharmacokinetic properties of compound **30** in rats, dogs, and monkeys, and low systemic clearance, moderate volume of distribution, long terminal half-life and excellent bioavailability greater than 95% were observed in previous studies [29]. And no clinically significant chemistry, haematology, or ECG changes were observed. However, Incyte announced its decision not to initiate two 6-month Phase IIb trials for compound **30** in treatment-experienced HIV patients owing to business reasons on March 3, 2008 [70, 71].

Scientists from Novartis disclosed NIBR-1282 **31** as a selective and competitive CCR5 inhibitor [72]. It blocks the binding of MIP-1α to CCR5 (IC_{50} = 5.1 nM) with good oral bioavailability in cynomolgus monkeys and dogs, without showing any sign for cardiac side-effects even at a concentration of 10 μM. Novel piperidine derivatives were disclosed as CCR5 inhibitors by Gabriel and co-workers [73]. Among them, compound **32** is the most potent inhibitor with an IC_{50} value of 43 nM in antiviral assays. Similarly, Bourque and co-workers described a series of piperidine derivatives as CCR5 inhibitors (compounds **33-35**) [74]. These compounds are claimed to have IC_{50} values ranging from 0.01 nM to 50 μM in anti-HIV-1 assay using PBMC and R5.

2.5. 1,4-Disubstituted Piperidine Derivatives

Takeda discovered a novel 1,4-disubstituted piperidine lead compound **36** by HTS, which blocked the binding of RANTES to CCR5 (IC_{50} = 1900 nM) [75]. Subsequent optimization identified a series of 1, 4-disubstituted piperidine derivatives, exemplified by **37**, which has low nanomolar





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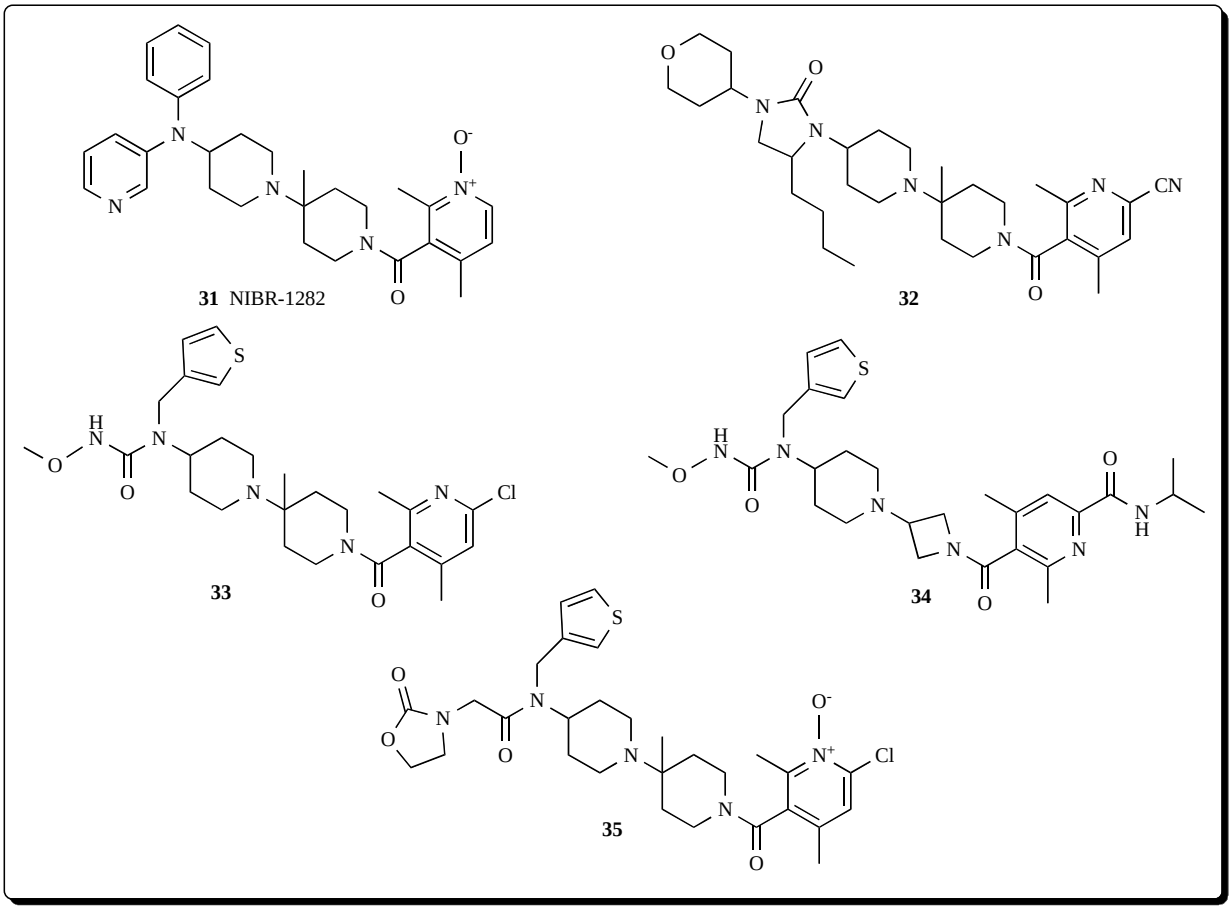

31 NIBR-1282

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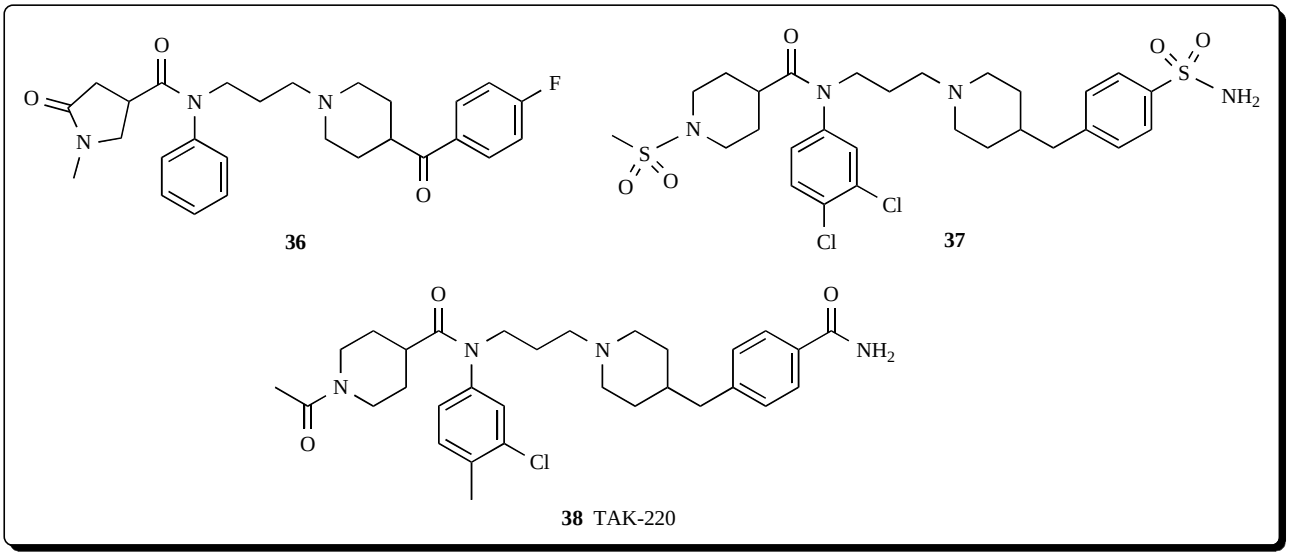
affinity for CCR5 and exhibits good anti-HIV-1 activity (blocks the binding of RANTES to CCR5 IC_{50} = 3.4 nM) [76]. However, *in vitro* metabolic stability studies showed these compounds can be rapidly metabolized. Further optimization by incorporating various polar groups led to the discovery of TAK-220 **38** [75], also known as TBR-220, which has entered Phase I clinical trials endorsed by Tobira.

TAK-220 potently inhibits the binding of RANTES (IC_{50} = 3.4 nM) and MIP-1α, yet it barely inhibits the binding of MIP-1β [77]. It shows potent inhibition of membrane fusion

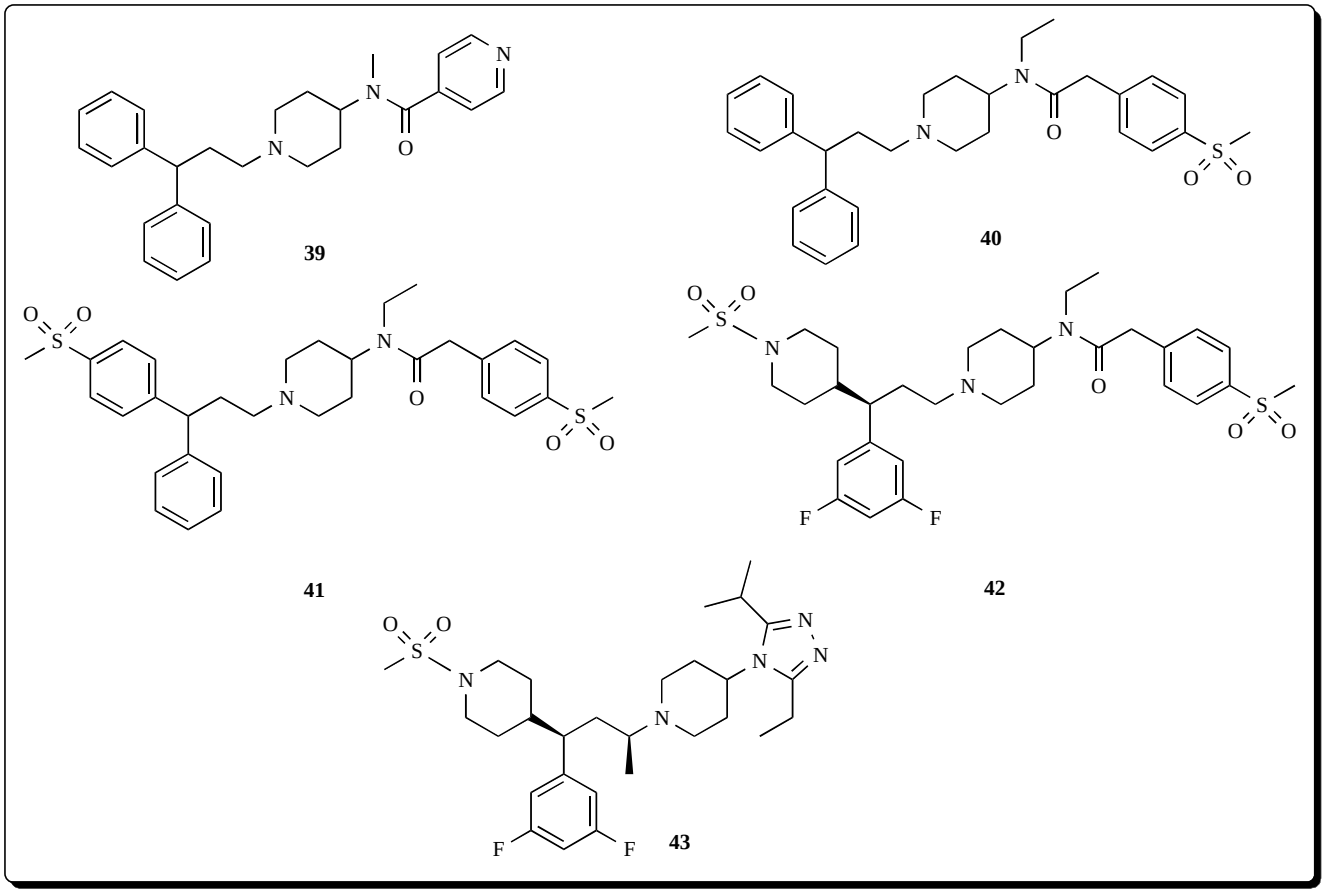


36 **37**

38 TAK-220

(IC_{50} = 0.42 nM) and strongly inhibits the replication of CCR5-using HIV-1 clinical isolates in human PBMC (mean EC_{50} =1.1 nM, EC_{90} =13 nM) [75]. At a dose of 5 mg/kg, **38** shows oral bioavailabilities of 9.5 and 28.9% in rats and monkeys, respectively [78]. Synergy of anti-HIV-1 between **38** and various antiretroviral drugs *in vitro* has been observed with all drugs at the 90 and 95% inhibitory concentrations [79]. The favorable drug interactions suggested that further clinical evaluation is warranted.

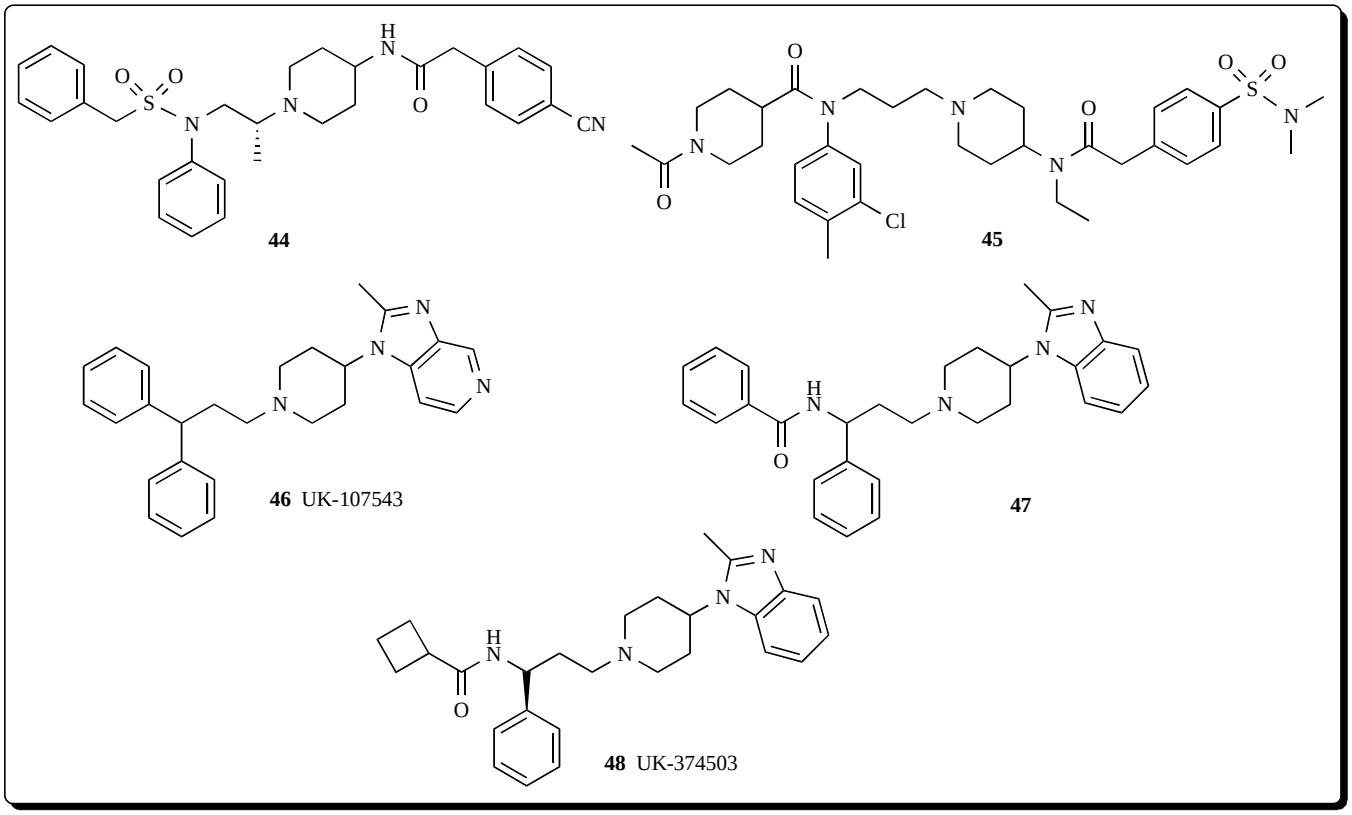
Researchers from AstraZeneca reported a series of 1,4-disubstituted piperidine derivatives **39-43**, with potent CCR5 inhibitory activity [80-83]. Compound **40**, derived from lead compound **39**, blocks the binding of RANTES to CCR5 (IC_{50} = 76 nM) [80], but the existence of human muscarinic and serotonergic activity indicate that further optimization is required. Addition of strongly electron-withdrawing substituents, such as methanesulfonyl group at 4-position of phenyl ring (compound **41**), resulted in a dramatic increase



39 **40**

41 **42**

43



of potency (IC_{50} = 1.7 nM) [81]. In an effort to further improving potency, replacing phenyl ring to methanesulfonyl-piperidine ring resulted in compound **42** with good affinity (IC_{50} = 0.3 nM) [82].

Compound **43**, an 3-ethyl-5-isopropyl-4*H*-1,2,4-triazol-4-piperidine derivative also reported by AstraZeneca [83], blocks the binding of MIP-1β to CCR5 (IC_{50} = 6.5 nM). The oral pharmacokinetic property of **43** in rat is not well. Although it has low clearance (18 ml/min/kg), the volume of distribution (4.1L/kg) is small, terminal half-life (4.0 hr) is short, and the bioavailability is poor (28%).

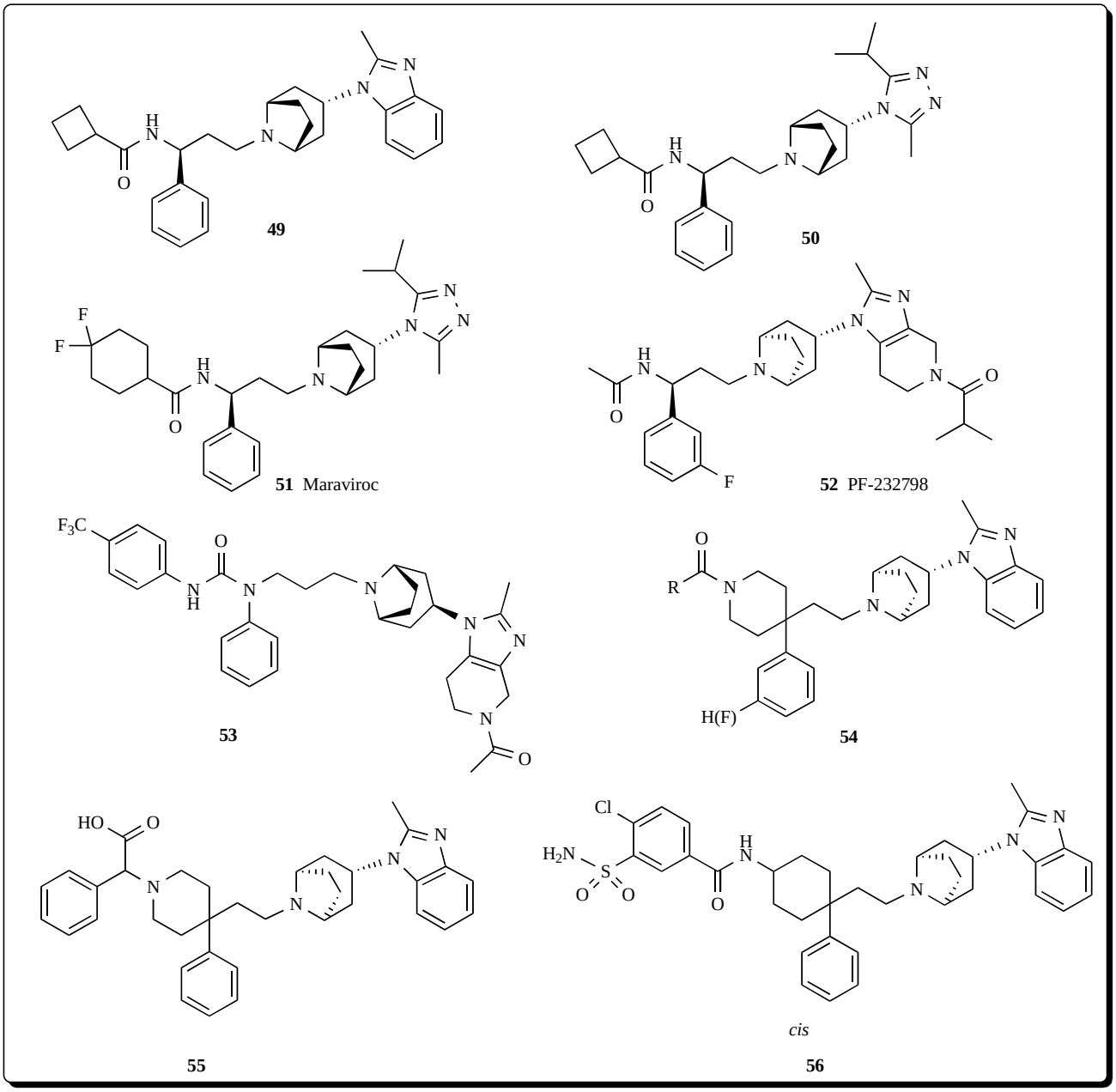
Huck and coworkers reported further modification on above compounds through the change of substitution pattern on the nitrogen atom and addition of a polar group between the two phenyl rings [84]. Compound **44**, the most potent compound in Huck's work, has an IC_{50} value of 0.5 nM in blocking the binding of MIP-1β to CCR5, and 1.9 nM in HIV-infection assay. Recently, Long and co-workers claimed a novel series of 1,4-disubstituted piperidine derivatives based on **40** and **38** [85], such as compound **45**, which shows an IC_{50} value of 2.5 nM in HIV-Infection assay.

HTS of the Pfizer Corporate compound library using inhibition of MIP-1β binding to the human CCR5 identified UK-107543 **46** with modest potency (IC_{50} = 0.4 μM) [86, 87]. Compound **46** is not an ideal lead because of its high lipophilicity and weak binding affinity. Replacing the imidazopyridine moiety with benzimidazole and introducing amide group afforded compound **47** (IC_{50} = 45 nM). Further studies of **47** showed that the polarity is increased compared with the lead compound. SAR investigation of different amide substituents led to the discovery of UK-374503 **48** (IC_{50} = 13 nM).

2.6. Tropane Derivatives

The CYP 2D6 inhibition of UK-374503 **48** is unacceptable and further SAR optimization indicated that tropane moiety could be an alternative for piperidine [29, 87]. This resulted in the discovery of compound **49** with no inhibition on CYP 2D6, excellent binding affinity and antiviral activity (MIP-1β IC_{50} = 6 nM; EC_{90} = 3 nM). Replacing the benzimidazole moiety of **49** with a substituted triazole gave compound **50**, whose lipophilicity is greatly reduced while antiviral activity maintains (IC_{90} = 29 nM), but its *h*ERG channel inhibition is increased compared with compound **49**. Therefore, scientists from Pfizer adjusted their strategy to modify the amide moiety to limit *h*ERG channel inhibition. This strategy ultimately led to the emergence of maraviroc (UK-427857, Trade name: Celsentri, Selzentry) **51**, which became the first CCR5 inhibitor approved by FDA in August 2007.

Maraviroc is a potent inhibitor of MIP-1β binding to CCR5 (IC_{50} = 2 nM) and a potent antiviral agent (EC_{90} = 1 nM for inhibition of HIV_{BAL} replication in PBMCs) [29, 87]. It is active against all R5-tropic HIV strains and demonstrates exquisite selectivity against other chemokine receptors, as well as a range of relevant enzymes, receptors, and ion channels, for instance, it shows no significant binding to *h*ERG channel (IC_{50} > 10 μM) and no significant inhibition (IC_{50} >100 μM) against any of the major CYP isoforms tested. Pharmacokinetic studies demonstrated that **51** is widely distributed with a V_d of 194 L, moderately bound to plasma proteins (75.5%) and shows absolute bioavailability



of a 300-mg dose (33%) [88]. The terminal elimination $t_{1/2}$ is approximately 17 h for multiple-dose regimens and does not alter significantly with dose [89]. In clinical studies, single doses of up to 900 mg and multiple doses of up to 300 mg bid for 28 days are all well tolerated. R5-treatment-naive patients who received **51** monotherapy at multiple doses (from 25 mg daily to 300 mg twice daily) for 10 days experienced a viral load reduction of up to 1.64 $\log_{10}$ copies/ml and a maximum viral load reduction of up to 1.84 $\log_{10}$ copies/ml [30].

MOTIVATE (maraviroc plus optimized therapy in viremic antiretroviral treatment-experienced patients) 1 and 2 studies were conducted to evaluated the efficacy and safety of adding maraviroc (either once or twice daily) to OBT in treament-experienced patients infected with CCR5-tropic HIV [71, 90]. One of virologic responses in those who achieved HIV-1 RNA of less than 50 copies/ml at 48 weeks in MOTIVATE 2 is 16.7, 43.2 and 45.5% in the placebo, maraviroc once-daily and twice-daily, respectively (both $p < 0.0001$). In addition, CD4 count recovery is greater with maraviroc once or twice daily than with placebo (both $p < 0.0001$).

The efficacy of maraviroc in treatment-naive patients was evaluated in the MERIT (maraviroc versus efavirenz regimens as initial therapy) study, comparing maraviroc with efavirenz, each in combination with zidovudine and lamivudine. Patients with only CCR5-tropic HIV and HIV RNA concentration >2000 copies/ml were randomized to zidovudine/lamivudine with either efavirenz or once/twice-daily maraviroc. The once-daily maraviroc arm was closed because of failures to meet prespecified noninferiority criteria. The twice-daily maraviroc arm and efavirenz arm dem-

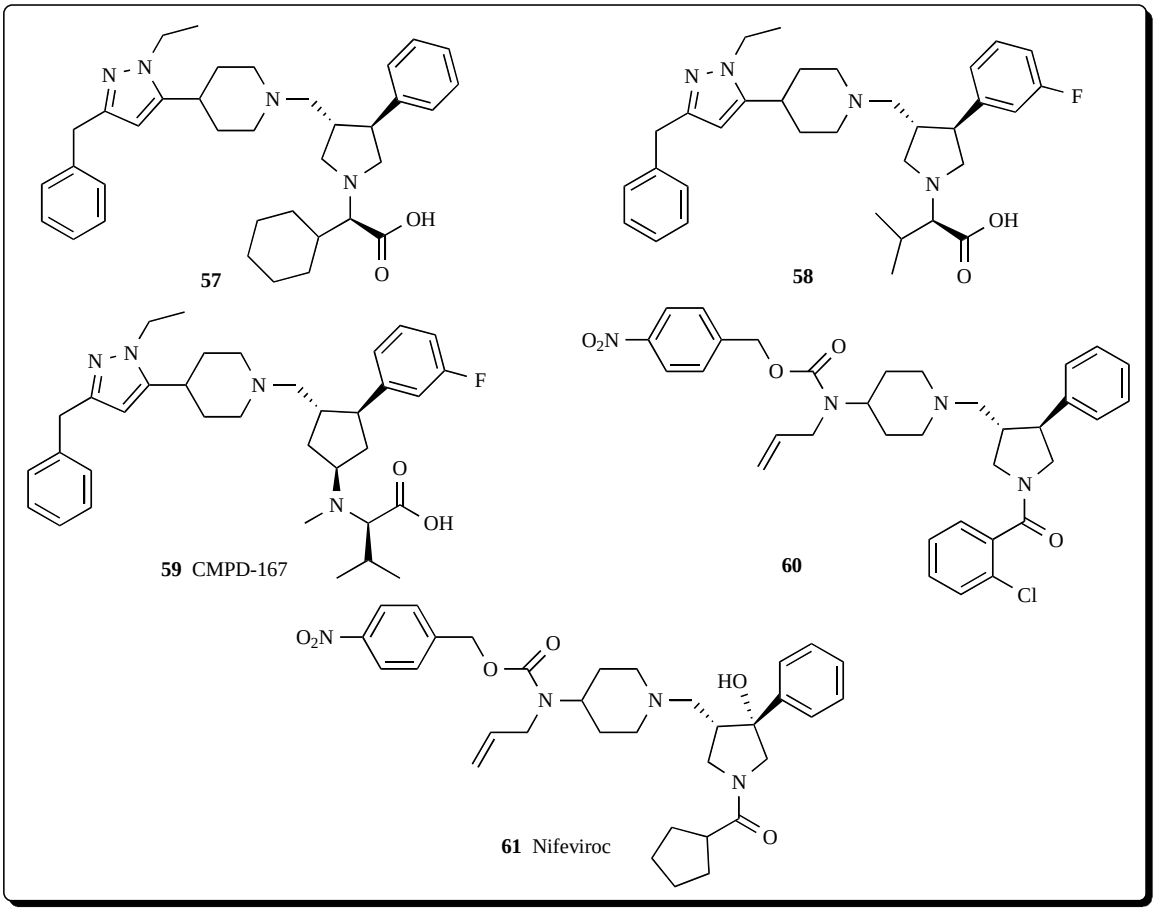
onstrated similar results with 69.3% *vs.* 65.3% of patients having < 50 HIV-1 RNA copies/ml. However, this small difference in response did not meet the predefined 10% criteria for noninferiority [71, 90]. Based on these results, Pfizer was continuing Phase III clinical trials to evaluate maraviroc for the treatment of antiretroviral treatment-naive patients harbouring CCR5-tropic HIV-1 [31].

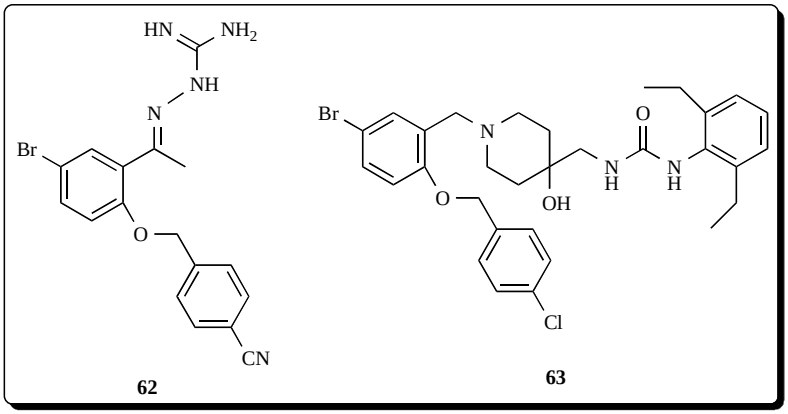
PF-232798 **52**, identified in a medicinal chemistry programme guided by screening approximately 3000 compounds, is an imidazopiperidine-tropane small molecular inhibitor developed by Pfizer following the successful licensing of maraviroc. Preclinical and clinical data demonstrated that it is highly selective for CCR5, very similar potencies to maraviroc in antiviral activity assay (EC_{90} = 2 nM), and potent broad-spectrum anti-HIV-1 activity. In Phase I Clinical trial, **52** was well tolerated in normal volunteers at the 250 mg dose once-daily and exhibited more favorable pharmacokinetic than **51** [91]. It is now in Phase II clinical trial. Justin and co-workers identified another series of substituted imidazopiperidine-tropane CCR5 inhibitors [92]. Compound **53** shows attractive antiviral property (IC_{90} = 6 nM) and an attractive pharmacokinetic profile (52% oral bioavailability and a terminal half-life of 10.7 h). **53** is selective and showed no significant inhibition against other chemokine receptors when tested up to a concentration of 10 μM.

Researchers at GlaxoSmithKline disclosed several series of CCR5 inhibitors through derivatization at the *N*-terminal of the piperidine ring in the tropane derivatives [93], originating from a class of comounds exemplified by **54**. Amino acids series appeared promising, because they retained high antiviral activities and minimized *h*ERG/QTc liabilities. Among them, compound **55** has an IC_{50} of 8 nM in the MIP-1β binding assay and 9.7 nM in the antiviral activity assay, but it has no oral exposure due to extremely high clearance and potentially poor permeability [94]. To explore the scope of the piperdine ring system and diversify current scaffolds [95], the piperdine ring was replaced with cyclohexylamine and *cis*-C0-C3 linker analogues were synthesized between the cyclohexyl and the tropane rings to determine the optimum length of the linker. Compound **56**, a representative of this series, demonstrates a highly potent inhibition against HIV-1 virus infection in the HOS cell assay (IC_{50} = 58 nM).

2.7. Pyrrolidine Derivatives

Merck provided a series of conformationally locked 1,3,4-trisubstituted pyrrolidine derivatives represented by compound **57** [96], displaying an IC_{50} value of 1.2 nM in the MIP-1β binding assay and 0.4 nM *in vitro* antiviral activity assay. However, compounds of this series lack satisfactory pharmacokinetic profile and off-target selectivity. Herein they modified the alkyl acetic acid portion and the phenyl on pyrrolidine of compound **57** to afford the isopropyl compound **58**, which shows high antiviral activity *in vitro* (IC_{50} = 3.7 nM), excellent selectivity, and good oral bioavailability. Further SAR extended the pyrrolidine group to trisubstituted cyclopentanes giving rise to compound **59**,



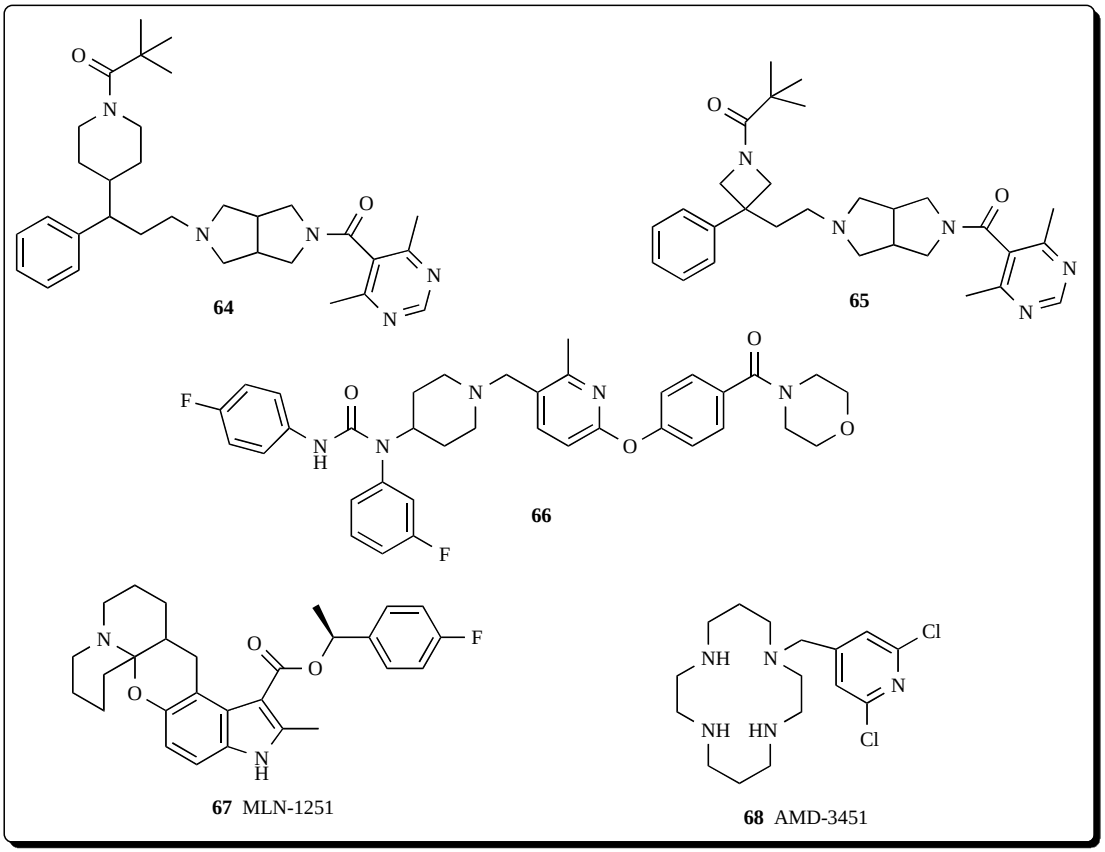


known as CMPD-167 (MRK-167) [97]. It shows significant antiviral activity (IC_{95} < 8 nM), and the oral bioavailability is reported between 43-66% in rats, dogs, and monkeys [67]. CMPD-167 has been licensed to International Partnership for microbiocides as a topical intravaginal gel to protect women from HIV, because it shows the ability to protect macaques from infection by a R5 strain of SHIV [98].

Ma and co-workers designed a series of 1,3,3,4-tetrasubstituted pyrrolidines as potential new CCR5 inhibitors based on compound **60** [99, 100]. Introduction of a hydroxy group at the 3-position of the pyrrolidine ring (Nifeviroc (TD-0232), compound **61**) not only made enantioselective synthesis of the core structure easier, but also resulted in compounds with good binding affinity, potent anti-HIV activity, and promising oral bioavailability. Nifeviroc blocks the binding of RANTES to CCR5 with an IC_{50} value of 2.9 nM, and inhibits the replication of seven genetically diverse, R5 HIV-1 isolates or laboratory strains in PBMC with EC_{50} values ranging from 0.3 nM to 30 nM. Oral bioavailability is assessed and determined to be 41.2 % and 21.6 % in rats and dogs, respectively. Compound **61** has entered into Phase I clinical trial by TargetDrug corporation in China collaborating with Avexa corporation.

2.8. Benzyloxybenzene Derivatives

Berlex identified several benzyloxybenzene derivatives by HTS against an internal compound library using a MIP-1α binding assay [101]. Compound **62** exhibits the highest activity with an IC_{50} value of 93 nM in the MIP-1α binding



assay. Meanwhile, the guanylhydrazone moiety was not required for CCR5 inhibition potency. Further investigation led to the replacement of the guanylhydrazone with a tertiary amine for improving the potency and exploring the pharmacokinetic profile [102]. This change resulted in a potent CCR5 inhibitor, compound **63**. It blocks MIP-1α binding to CCR5 with an IC_{50} value of 11nM. The pharmacokinetics study in rats revealed that compound **63** has high clearance (60 mL/min/kg), elevated volume of distribution (22 L/kg) and the tendency to be metabolized by HLM. Because of its poor pharmacokinetic properties, further work on this series has been terminated.

2.9. Other Small-Molecule CCR5 Inhibitors

There are other kinds of small-molecule CCR5 inhibitors reported in literature. For example, Roche reported a novel series of octahydropyrrolo[3,4-c]pyrrole derivatives as CCR5 inhibitors [103, 104]. Compounds **64** and **65** exhibit IC_{50} values of 8.2 nM and 27 nM in RANTES binding assay, respectively. A series of novel piperidine derivatives were claimed by Smithkline Beecham [105]. The IC_{50} value of the representative compound **66** is less than 10nM in antiviral assay. Magnus Rnn *et al* developed an expedient synthesis of MLN-1251 **67**, a CCR5 inhibitor for the treatment of HIV [106], but no specific bioactivity has been reported.

AMD-3451 **68**, a *N*-pyridinylmethyl cyclam derivative, has been reported as a CCR5/CXCR4 inhibitor [107]. It shows antiviral activity against a wide variety of R5, R5/X4 and X4 strains of HIV-1 and HIV-2 in the micromolar concentration range (IC_{50} ranging from 1.2 to 26.5 μM), and inhibits R5, R5/X4 and X4 HIV-1 primary clinical isolates in PBMCs (IC_{50}: 1.8-7.3 μM). Meanwhile, this compound does not interact with other chemokine receptors, and this profile would be important for the development of an effective anti-HIV agent.

3. CONCLUSION

From what has been discussed above, it is clearly shown that inhibiting CCR5 is a rational biochemical approach to prevent HIV infection. The discovery and development of small-molecule CCR5 inhibitors is currently a hotspot in the field of HIV therapy. Most CCR5 inhibitors identified up till now are discovered through HTS. Guided by detailed SAR studies and optimization efforts, various highly potent small-molecule inhibitors have been synthesized and advanced into clinical trials. More encouragingly, maraviroc has approved by FDA as the first small-molecule CCR5 inhibitor. However, various challenges still exist, such as target validation, allosterism, viral resistance and tropism, together with possible long term adverse efforts. Thus, development of novel CCR5 inhibitors overcoming the above disadvantages is still the focus of extensive research works.

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