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Review Article

Non-peptidic Chemokine Receptors Antagonists as Emerging Anti-HIV Agents

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HIV entry within the cell involves the presence of at least two chemokine co-receptors, the CCR5 and CXCR4 receptors. Viral entry can be inhibited by the natural ligands for CXCR4, the CXC chemokine SDF-1 and CCR5, the CC chemokines RANTES, MIP-1α and MIP-1β, respectively. Much research has been devoted ultimately to the development of small molecule chemokine antagonists that inhibit virus entry within the cell, and constitute in this way novel antiviral medications. The most potent and specific CXCR4 antagonists reported up to now are the bicyclam derivatives, which also potently block X4 HIV replication. One such compound, AMD3100 has proved to be a highly specific CXCR4 antagonist, which consistently blocks the outgrowth of all X4 HIV and dual-tropic (R5/X4) variants that use CXCR4 for entering the cells. From such bicyclam analogues, AMD3100 was selected as the clinical candidate, which, after initial Phase I studies, proceeded to Phase II trials, but unfortunately showed significant cardiac side effects which lead to its withdrawal from further development. The first non-peptidic compound that interacts with CCR5, but not with CXCR4, is a quaternary ammonium derivative, TAK-779, which also shows potent but variable anti-HIV activity. A large number of potent CCR5 antagonists from several classes of polycyclic derivatives have been recently disclosed. Many such derivatives showed nanomolar binding affinity to the receptor, and at least one of them, the oxime–piperidine derivative SCH-351125 has progressed to clinical evaluation. The development of such agents for clinical use may constitute an additional approach for the treatment of HIV infection, in addition to the classical one involving reverse transcriptase and protease inhibitors.

Keywords: Antiviral agent; Bicyclam; Chemokine receptor; CCR5; CXCR4; HIV entry

INTRODUCTION

HIV entry within the target cells involves a series of molecular events that started to be understood in detail in the last decade. The T-lymphocyte cell surface protein CD4 is the primary receptor involved in the interaction with the viral glycoprotein gp120, but a cellular co-receptor is also needed for the successful entry of the virus within the cell.[1–4] At least two types of such co-receptors have been identified so far, both belonging to the chemokine family of seven-transmembrane-spanning receptors coupled to a G-protein signaling pathway:[5–8] the CC chemokine receptor 5 (CCR5) (which binds the chemotactic chemokines, the monocyte inflammatory protein (MIP)-1α, and MIP-1β, and RANTES [**r**egulated upon **a**ctivation **n**ormal **T**-cell **e**xpress and **s**ecreted]) and the CXC chemokine receptor 4 (CXCR4) (which binds the stromal derived factor (SDF)-1 as ligand).[1–8] These receptors therefore are the gateways for HIV entry, determinants of viral tropism and sensitivity. The CCR5 receptor is used by macrophage (M)-tropic viruses and CXCR4 is used by T-lymphocyte (T)-tropic virus.[1–8]

The three main steps of virus entry within the cell can be detailed as follows:

(i) **Attachment of the virus to the host cells**. This process involves the formation of a complex between the trimeric gp120–gp41 viral glycoproteins, the CD4 receptor and the chemokine co-receptor (CCR5 or CXCR4).[2,7]

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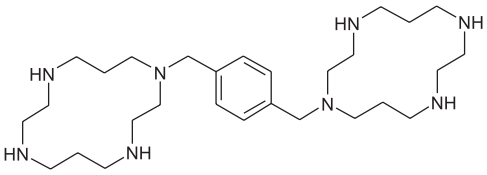
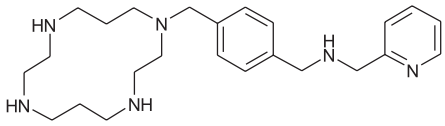
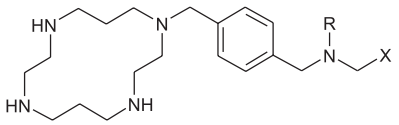


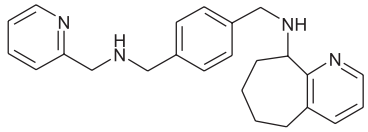
1: AMD3100



2: AMD3465



3: R = H, Me; X = 2-, 3- or 4-pyridyl, thienyl, aminophenyl; etc.



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(ii) **Interaction of the virus with the co-receptors**. The amino-terminal fusion peptide of gp41 is inaccessible in the native state, but following interaction of gp120 with the CD4 protein, a conformational change occurs, leading to exposure of the gp120 third hypervariable (V3) domain loop, with insertion of the aminoterminal peptide into the target cell membranes, *via* a "prehairpin" intermediate.[2,7]

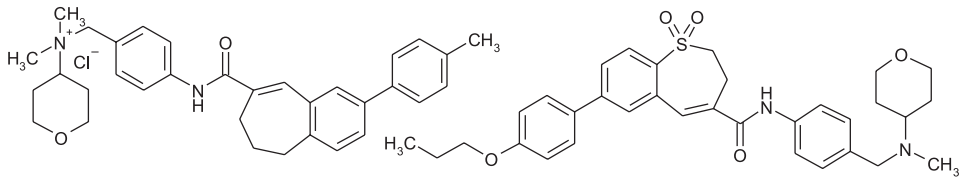
(iii) **Fusion of the virus and host cell membranes**. Intramolecular interactions between the C- and N-terminal peptide regions of gp41 lead to the formation of a hairpin configuration (actually a trimer of hairpins), which is followed by juxtaposition of the host cell and viral membranes, i.e. membrane fusion.[2,7,9]

Considering the complexity of the molecular events briefly outlined above, it can be envisaged that all of these three steps have been considered for the drug design of HIV entry inhibitors.[1–3] Indeed, several approaches have been reported ultimately of agents that interact with one of the steps mentioned above, such as for example: fusogenic particle peptide antagonists (agents of peptidic nature that interact with the gp120/gp41–CD4–chemokine receptor interaction);[1–3,10] chemokine and chemokine derivatives as fusion inhibitors;[3] peptide-based antagonists of the CXCR4 receptors[3,11] as well as small molecule chemokine antagonists of either CXCR4 or CCR5 receptors.[3,12–18] Since the entire field of HIV—cellular fusion inhibitors has recently been reviewed,[1,2] in this paper we shall deal only with the small molecule antagonists of the chemokine receptors (CXCR4 and CCR5) involved in HIV pathogenesis. The present review is intended to present the last developments in the design of such chemokine antagonists, since several groups have started impressive research programmes in this field and many interesting compounds have now been disclosed, which may represent major steps for the clinical development of HIV entry inhibitors as antiviral agents.

CXCR4 RECEPTOR ANTAGONISTS

AMD3100 **1** was the first chemokine antagonist to enter clinical studies for the treatment of AIDS/HIV infection (the compound arrived in Phase II clinical studies, but since then its development has been arrested, see later in the text).[15,18,19,20] AMD3100 is a bicyclam derivative possessing strong anti-HIV activity due to its inhibition of the viral protein—CXCR4 interaction, with an IC_{50} of 2–20 nM (depending on the viral strain).[2,19] This compound is active only against T-lymphocyte-tropic CXCR4-using viruses, and inactive against CCR5 or M-tropic viruses.[19] Detailed pharmacological data on the bicyclams will be not provided here, but they can be found in the excellent review by De Clercq.[19] We should stress instead the structure–activity relationship for this novel class of antiviral agents.

Thus, recently, additional derivatives belonging to this class, such as compounds **2** and **3**, have been reported, possessing only one cyclam moiety in their molecule, as compared to the bicyclam lead **1**.[20] Probably such compounds may show a better pharmacological profile since due to the highly polar nature of AMD3100, this compound must be administered parenterally, as its bioavailability via oral administration is very low, and this may constitute an undesired complication for the therapy. Presumably, compounds with lower molecular weights and only one cyclam moiety may possess improved pharmacological features. Indeed, AMD3465 (**2**) and several congeners of type **3**



5: TAK-779

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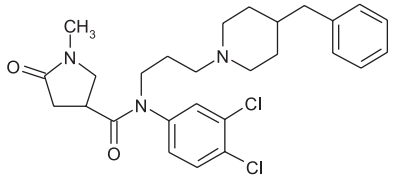

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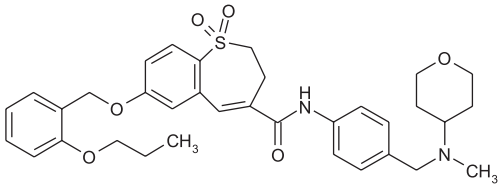

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possessing different heterocyclic/aromatic moieties in their molecules, show strong antiviral properties, with EC_{50} values in the range of 0.008–0.20 μg/mL.[20] Similarly to AMD3100, these new compounds inhibit SDF-1α (signal transduction) induced calcium flux upon binding to the CXCR4 receptor, inhibiting in this way the virus penetration within the cell.[20]

The lead **1** was further modified,[20] leading to bisamines such as **4**, in which only the central 1,4-bisphenylene moiety has been preserved, whereas different heterocyclic groups have been attached to the two terminal positions. Such moieties include: at least one 2-pyridyl moiety (such as in derivatives **2**–**4** mentioned above), together with a bulkier group, an example of which is shown in structure **4** (but a large variety of such heterocyclic moieties have been incorporated in these compounds, sometimes substituted with arylsulfonamide, ureido, methoxyaryl or cyanophenyl groups).[19,20] The mechanism of action and the antiviral activity of these new derivatives seem to be of the same type as those of the structurally related AMD3100 and AMD3465, but compounds **4** should presumably possess a better bioavailability when given orally, due to their less polar character, as compared to the cyclam/bicyclam containing compounds mentioned above.[19,20]

Unfortunately, due to cardiological problems in some patients treated with AMD3100, this compound was recently withdrawn from clinical development as an anti-AIDS drug.[3]

SMALL MOLECULE CCR5 RECEPTOR ANTAGONISTS

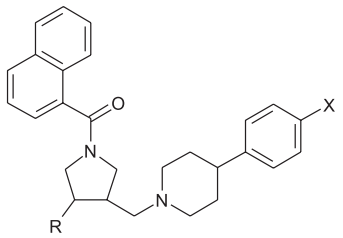
Searching for small molecule CCR5 antagonists by high-throughput screening (HTS) using RANTES and Chinese hamster ovarian (CHO)/CCR5 cells, led Takeda researchers to report[21] several lead molecules incorporating quaternary ammonium or quaternary phosphonium moieties, which was subsequently followed by the synthesis of a large series of anilides, among which was TAK-779 (**5**).[21,22] This compound is a highly potent and selective CCR5 antagonist, with an IC_{50} of 1.4 nM (in the binding assay). TAK-779 strongly inhibited the replication of M-tropic HIV-1, with EC_{50} values in the range of 1.2–3.7 nM.[21,22] The binding pocket of the CCR5 receptor for TAK-779 has also recently been identified,[23] and this compound is in preclinical evaluation as an antiviral agent.[21,22]

The charged nature of TAK-779 constitutes a problem from the pharmacological point of view, since such cationic derivatives tend to be membrane-impermeant,[24,25] and thus antivirals of this type should be administered only parenterally. This is the reason why Takeda continued to search for other types of CCR5 antagonists, devoid of positively-charged moieties. Some compounds of this type, such as **6**–**8** have recently been reported. Detailed antiviral data for these new derivatives have not yet been disclosed, but they should be much more membrane permeable as compared to the lead molecule, TAK-779.[3,21,22]

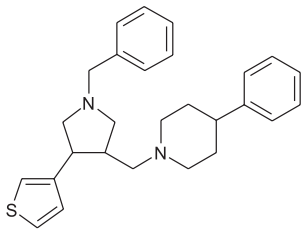
A large number of chemokine antagonists with anti-HIV activity has been discovered by the Merck group, with several promising leads emerging from detailed SAR studies.[12,13,26–31]

The main structural element of many compounds with such an activity consists of a 1,3,5-trisubstituted five-membered ring, usually of the pyrrolidine (such as in **9**–**14**) or cyclopentane (such as in **15**–**18**) type.[26–28]

Generally the three substituents of the central five-membered ring are bulky, and they include a large



9 R = t-Bu,c-C_3H_5, COOH
X = H, F



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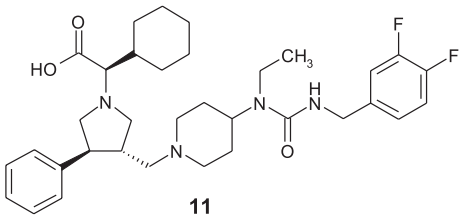

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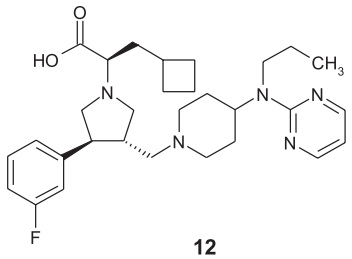

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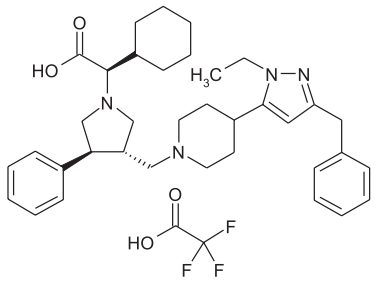

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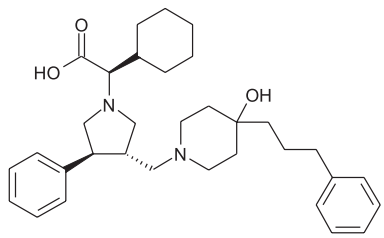

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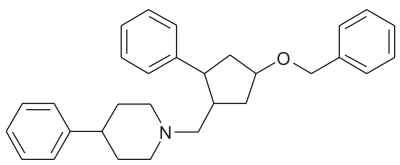

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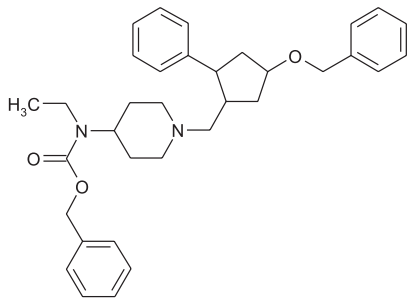

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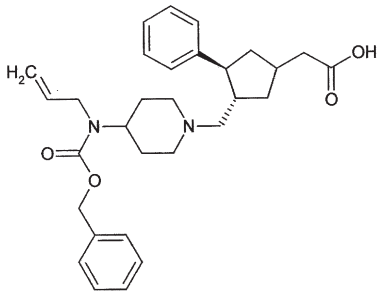
variety of moieties, such as: (substituted)-phenyls/-naphthyls; five/six-membered heterocyclic groups; 4-substituted piperidines; arylsulfonamides, etc., in a great variety of combinations and stereochemistries, as seen in structures **9**–**18** above. Many of the target compounds bind to the CCR5 receptor with affinities of $<1\,\mu M$. In addition to CCR5, these compounds also bind the chemokine receptor CCR3, but no detailed data regarding selectivity/specificity for one of these two receptors have been provided.[26–28]

Much more detailed data were published for the substituted 2-aryl-1-[N(methyl)-N-(phenylsulfonyl)amino]-4-(piperidin-1-yl)butanes **19**[12,13] and the related derivative **20**[13] by Finke's group, compounds which are totally different from the structural point of view from the previously disclosed CCR5 antagonists **9**–**18** mentioned above. These compounds have been identified as CCR5 antagonists through an extensive screening programme of Merck's collection of derivatives, using a HTS binding assay of labeled-MIP-1α to stably expressed human CCR5 receptors in CHO cells.[12] A number of sulfonamides possessing 1-(N-alkyl-N-phenylsulfonylamino)-2-(3,4-dichlorophenyl)-4-(piperidin-1-yl)butane moieties were found active in this way, whereas the corresponding carboxamides were devoid of CCR5 antagonistic activity.[12] The synthetic efforts were concentrated on such lead molecules eventually led to the spiro-2,3-dihydrobenzthiophene-3,4′-piperidin-1′-yl derivative **19** (X = Cl)—as



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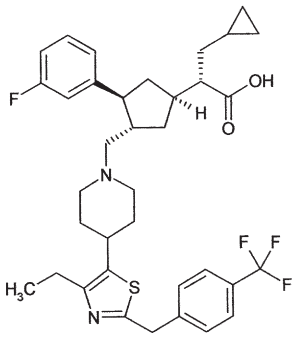

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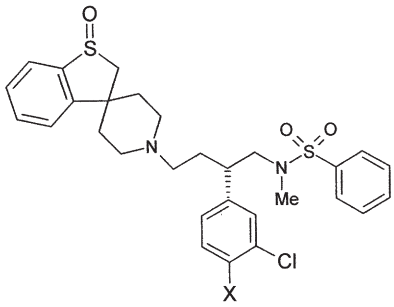
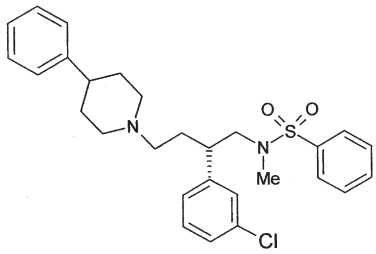


19: X = Cl, H



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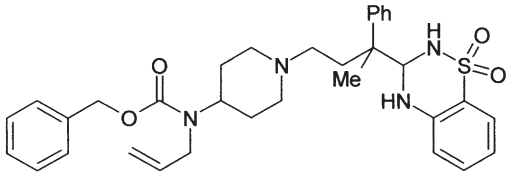

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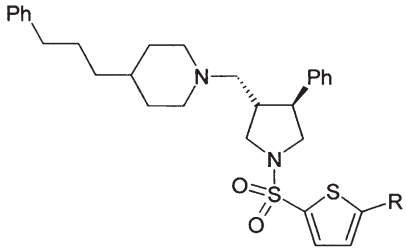
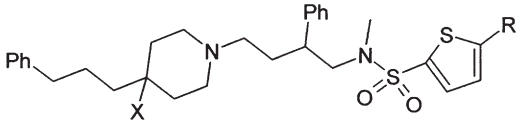
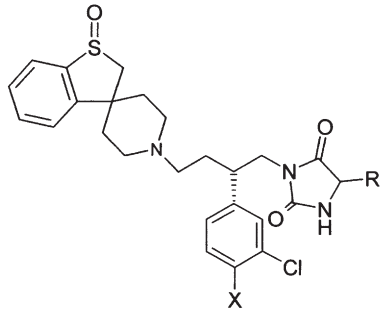


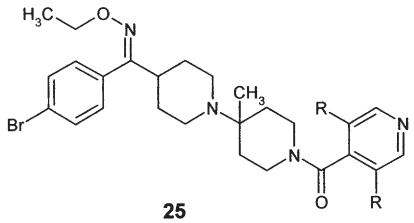
22: R = H, 2-pyridyl.



23: R = H, 2-pyridyl; X = H, OH.



24: R = Ph, $PhCH_2$, etc.



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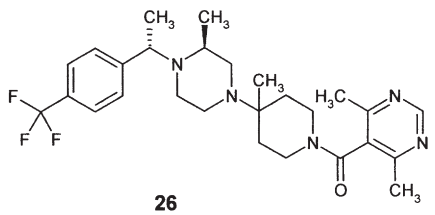

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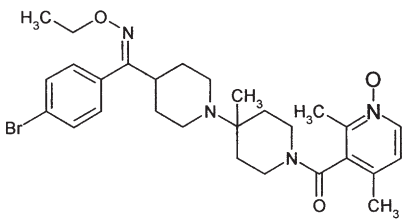
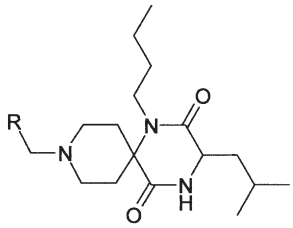
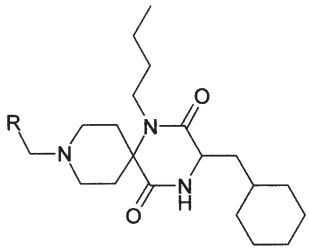
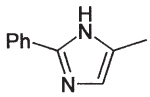


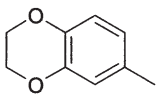
27: SCH-351125



28: R = $Ph(CH_2)_5$
29: R = $Ph\text{-}O\text{-}C_6H_4$



30: R =  (E913)

31: R = 

sulfoxide—showing a nanomolar affinity for the CCR5 receptor ($IC_{50} = 35$ nM), without appreciable binding to the related CCR1, CCR2 and CCR3 receptors (IC_{50} values over 1000 nM).[12] Subsequent modification of the arylsulfonamide moiety by a large variety of alkyl, substituted aryl or hetaryl moieties were detrimental to the binding affinity, and the phenylsulfonamide group of **19** has been preserved as such. The halogeno-phenyl moiety of the lead **19** has also been extensively modified, but generally the obtained derivatives were less active then the dichloroderivative (except for the 3-chlorophenyl-derivative, **19** (X = H)[13]). The spiro-2,3-dihydrobenzthiophene-3,4′-piperidin-1′-yl moiety of **19** has been replaced by the 4-phenyl-piperidine moiety present in **20**, leading to a compound with slightly improved binding affinity for the CCR5 receptor ($IC_{50} = 30$ nM for **20**).[13] Further synthetic work by the same group led to some more potent CCR5 antagonists, such as **21** (its *R*-isomer has an IC_{50} of 18 nM)[29] and **22–24** (discovered by a combinatorial chemistry approach).[30,31] Some of these last derivatives are indeed excellent and selective CCR5 antagonists, possessing IC_{50} values in the range of 2–20 nM.[29–31]

Researchers from Schering reported a large series of piperidine and piperazine derivatives of types **25** and **26**, with potent CCR5 antagonistic activity.[32–35] These compounds possess the piperidine/piperazine moieties present in some of the Merck derivatives, but their structure is in fact completely original, and they seem to be much more potent CCR5 antagonists as compared to the Merck derivatives: in fact some of the Schering compounds inhibit RANTES binding to the CCR5 receptor with K_I-s in the very low nanomolar range (0.1–3 nM), making them some of the most tight-binding CCR5 antagonists reported up to now.[32–34] One of the N-oxides derived from the lead structure **25**, SCH-351125 **27**, specifically inhibits HIV-1 infection mediated by CCR5 in U-87 astroglioma cells but has no effect on infection of CXCR4-expressing cells.[35] SCH-351125 has broad and potent antiviral activity *in vitro* against primary HIV-1 isolates that use CCR5 as their entry co-receptor, with mean IC_{50} values ranging between 0.4 and 9 nM. Moreover, SCH-351125 strongly inhibits the replication of an R5-using HIV-1 isolate in SCID-hu Thy/Liv mice. SCH-351125 has a favorable pharmacokinetic profile in rodents and primates with an oral bioavailability of 50–60% and a serum half-life of 5–6 h. On the basis of its novel mechanism of action, potent antiviral activity, and *in vivo* pharmacokinetic profile, SCH-351125 is a promising new candidate for therapeutic intervention in HIV infection.[35]

Novel low molecular weight spirodiketopiperazine derivatives of types **28–31**, which potently

inhibit R5-tropic HIV-1 isolates through their antagonistic effects on CCR5 were recently reported by Maeda *et al.*[36] One such compound E913 (**30**) specifically blocked the binding of MIP-1α to CCR5 (IC_{50} of 2 nM) and MIP-1α-elicited cellular calcium mobilization (IC_{50} of approximately 20 nM). E913 potently inhibited the replication of laboratory and primary R5 HIV-1 strains as well as various multi-drug-resistant monocyte/macrophage tropic (R5) HIV-1 at IC_{50} values of 30 to 60 nM.[36] E913 was inactive against T cell tropic (X4) HIV-1; however, when combined with the CXCR4 antagonist **1** (AMD-3100), E913 potently and synergistically inhibited the replication of dualtropic HIV-1 and a 50:50 mixture of R5 and X4 HIV-1.[36] Antagonism in anti-HIV-1 activity was not seen when E913 was combined with the reverse transcriptase inhibitor zidovudine or protease inhibitors. E913 proved to compete with the binding of antibodies to CCR5 which recognize the C-terminal half of the second extracellular loop of CCR5.[36] E913 and its analogs were acid-resistant and orally bioavailable in rodents, proving that spirodiketopiperazine derivatives may be further developed as potential therapeutics for the management of HIV-1 infection.[36]

CONCLUSIONS

Although much progress has been registered ultimately in the treatment of viral diseases and especially HIV infection[37–41] by the use of highly active antiretroviral therapy [HAART, a combination of nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and/or aspartic protease inhibitors (PIs)], the massive viral replication, with more than 10^9 virions produced daily, and the high error rate of the reverse transcriptase, leads to the emergence of drug resistant strains and the stringent need of new therapeutic approaches. The use of small molecule chemokine antagonists of the type mentioned here might be one of the most important new strategies for achieving such a goal, with the potential of eradicating infection from tissues difficultly accessible to the presently available drugs (such as the lymph nodes, testes, CNS, etc.).[37–41]

Several interesting low molecular weight lead molecules have recently been reported as CXCR4 antagonists. Among the first type of such derivatives, the bicyclam AMD3100 **1** (from AnorMed) as well as some new generation mono-cyclams, show very potent anti-viral activity. Many CCR5 antagonists on the other hand have started to be reported only recently, with several highly potent antagonists disclosed by researchers from several drug houses. Although clear-cut structure–activity relationships are difficult to envisage at the moment, many of thestrong CCR5 antagonists reported so far possess a central ring system (five- or six-membered), generally trisubstituted with rather large and bulky moieties. The presence of sulfonamide groups in many such derivatives seems also to be beneficial for the binding affinity of the compound to the receptor. The conclusion of this review is that this is a very rapidly evolving field, with many new very potent chemokine antagonists being constantly reported, which hopefully will lead to the development of novel types of effective antiviral drugs in the near future.

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