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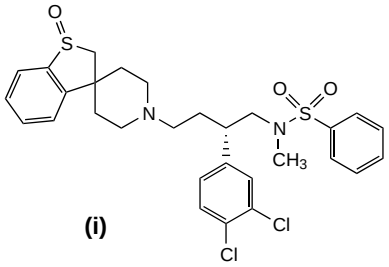
Novel antiviral molecules

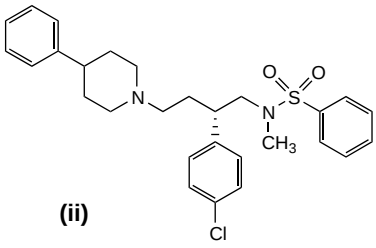
CCR5 antagonists as anti-HIV agents

Antagonists of the CCR5 chemokine receptor have attracted a lot of attention as potential anti-HIV agents. This is because HIV-binding to this receptor is required for viral entry and infection. Although the CXCR4 chemokine receptor also has a role in HIV-infection, it is CCR5 that has garnered most of the attention because HIV-1 variants that recognize this receptor predominate during early stages of infection. In fact, viruses that exclusively rely on the CXCR4 receptor for entry are rarely isolated from patients.

Two papers from researchers at Merck (Rahway, NJ, USA) describe the identification and SARs of a series of compounds that bind CCR5 and prevent infection by HIV[1,2]. In the first report, compounds similar to **(i)** were identified through screening of the corporate compound collection. This compound inhibits the binding of macrophage inflammatory protein-1α (MIP-1α) to the CCR5 receptor with an IC_{50} value of 35 nM, thereby inhibiting viral replication in tissue culture with an IC_{95} value of 6–12 μM. Initial SAR studies of this chemotype revealed that the spirocyclic sulfoxide could be simplified, as in **(ii),** but the phenyl ring and the sulfonamide were sensitive to modification. The *N*-methyl-phenylsulfonamide appears to be optimal for this portion of the molecule because all attempts at isosteric replacement resulted in a loss in potency. Substitution of the phenyl ring had a dramatic effect on activity with the 3-monosubstituted phenyl ring and unsubstituted phenyl ring being preferred.



(i)



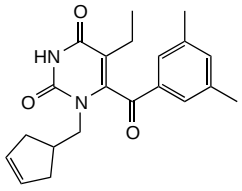
(ii)

1 Dorn, C.P. *et al.* (2001) Antagonists of the human CCR5 receptor as anti-HIV-1 agents. Part 1. Discovery and initial structure–activity relationships for 1-amino-2-phenyl-4-(piperidinyl-1-yl)butanes. *Bioorg. Med. Chem. Lett.* 11, 259–264

2 Finke, P.E. *et al.* (2001) Antagonists of the human CCR5 receptor as anti-HIV-1 agents. Part 2. Structure–activity relationships for substituted 2-aryl-1-(*N*-(methyl)-*N*-(phenylsulfonyl)amino]-4-(piperidin-1-yl)butanes. *Bioorg. Med. Chem. Lett.* 11, 265–270

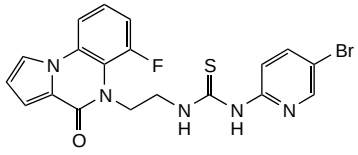
Non-nucleoside reverse transcriptase (NNRTI) inhibitors

A unique non-nucleoside reverse transcriptase inhibitor (NNRTI), SJ3366 (**iii**), has been developed by Buckheit and colleagues[3]. Although the compound is structurally related to the 1-[(2-hydroxyethoxy)methyl]-6-(phenyl-thio)thymine (HEPT)-based inhibitors, its uniqueness comes from the fact that it is active against both HIV-1 and HIV-2 and has a dual mode of action: inhibition of reverse transcriptase (RT) and inhibition of viral entry. The compound inhibits isolated RT derived from HIV-1, with a K_i value of 3.2 nM, but is inactive against RT from HIV-2. Its main mode of action against HIV-2, therefore, appears to be as an entry inhibitor. SJ3366 was evaluated against several laboratory and clinically isolated strains in established or fresh human cell preparations, yielding inhibition activities of 0.6–10 nM (HIV-1) and 3–480 nM (HIV-2) with little toxicity. Reduced activity was observed against HIV-1 strains possessing the Y181C, K103N and Y188C mutations in the RT enzyme, however, the compound selected for a virus having the Y181C mutation after five cell passages.

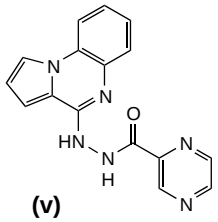


(iii)

In a separate study, quinoxalinylethylthioureas (QXPTs) such as compound **(iv)**, have been identified as potent, non-nucleoside inhibitors of HIV-1. Unfortunately, **(iv)** and related analogues display poor pharmacokinetic properties, having low oral bioavailability. In recent work by Campiani and coworkers[4], this chemotype is further explored to



(iv)



(v)

increase potency and enhance oral bioavailability. SAR studies generated several potent analogues based on the QXPT chemotype; however, like the parent compound, these displayed poor bioavailability. Presumably this is because of first-pass metabolism associated

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with the thiourea linker. Indeed, modifying this portion of the inhibitor as in **(v)** resulted in improved pharmacokinetics[4].

3 Buckheit, R.W. *et al.* (2001) SJ-3366, a unique and highly potent non-nucleoside reverse transcriptase inhibitor of human immunodeficiency virus type 1 (HIV-1) that also inhibits HIV-2. *Antimicrob. Agents Chemother.* 45, 393–400

4 Campiani, G. *et al.* (2001) Quinoxalinylethylthioureas (QXPTs) as potent non-nucleoside HIV-1 reverse transcriptase (RT) inhibitors. Further SAR studies and identification of a novel orally bioavailable hydrazine-based antiviral agent. *J. Med. Chem.* 44, 305–315

Human respiratory syncitial virus fusion protein

Although the medical community has been aware of respiratory syncitial virus (RSV) since its identification in 1955, it has only recently been appreciated that human RSV (hRSV) is responsible for the significant morbidity and mortality that occurs during annual outbreaks of infection. In fact, RSV appears to be the leading cause of lower respiratory tract infections in children and infants. RSV infection is a particular problem for infants in neonatal intensive care units, especially those born prematurely who experience bronchopulmonary dysplasia. RSV infection is also a problem for immunocompromised adults. In severe cases, standard treatment involves administering ribavirin. Until recently, ribavirin was the only available treatment but Synagis (MedImmune, MD, USA), an antibody-based therapeutic, has become available for use in passive immunization. However, these treatments are associated with side effects and high cost and, therefore, efforts are ongoing to find small-molecule-based therapeutics.

Recently, one promising target has been described by Zhao and colleagues[5] (Howard Hughes Medical Institute, Cambridge, MA, USA). They report the X-ray crystallographic characterization of two domains, termed HR-N and HR-C, of the RSV-F1 protein. The F1 protein is believed to be involved in virus binding and entry. The HR-N and HR-C domains form the fusion-protein core of RSV and are believed to enable viral entry through an interdomain association that 'pulls' the viral and cellular membranes into close proximity. Before this study it was predicted that association of these two domains was a crucial step in mediating the fusion of the viral and cellular membranes because analogous protein domains have been well characterized in several viruses, such as influenza, HIV-1 and Ebola. It was not surprising, therefore, that the X-ray structure shows that these two domains associate to form a trimer of hetero-HR-N–HR-C dimers, which is believed to be the fusion-active state.

More encouragingly, it has been found that peptides corresponding to the HR-C domain have been discovered that inhibit the virus[6]. This is analogous to HIV-1, where a similarly constructed protein based on the HIV fusion protein was found to inhibit viral entry[7]. These results, together with the structural characterization detailed in the current study, suggest that the likely mechanism of action for the HR-C-based inhibitor is as a viral-entry inhibitor. Moreover, because the HIV-entry inhibitor has been found to be useful in the clinic, it appears that an RSV-entry inhibitor designed in the same manner could lead to a clinically active therapeutic.

5 Zhao, X. *et al.* (2000) Structural characterization of the human respiratory syncytial virus fusion protein core. *Proc. Natl. Acad. Sci. U.S.A.* 97, 14172–14177

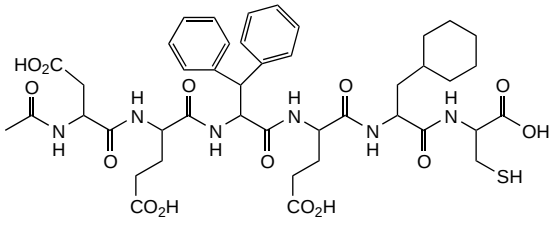
6 Lambert, D.L. *et al.* (1996) Peptides from conserved regions of paramyxovirus fusion (F) proteins are potent inhibitors of viral fusion. *Proc. Natl. Acad. Sci.* 93, 2186–2191

7 Chan, D.C. *et al.* (1998) HIV entry and its inhibition. *Cell*, 93, 681–684

Hepatitis C protease

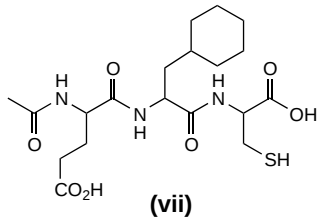
The HCV genome is expressed as a large polyprotein and, because of this, extensive processing is required during and after translation. The virus itself expresses two proteases, termed the NS2/NS3-protease and the NS3-protease, which have a role in this process. Of the two, the NS3-protease appears to be of greater interest because it, alone, processes the functional domain (i.e. the non-structural proteins) of the genome. For this reason the NS3-protease is considered a rather attractive target for anti-viral drug development, and several papers have appeared over the past few years describing potent substrate-based peptide inhibitors of this enzyme[8].

In addition to the protease domain NS3 also codes for a helicase/NTPase located in the C-terminal region. The majority of protease-inhibition studies reported to date employ a truncated form of NS3 containing only the protease domain. Inspired by the recent disclosure of the X-ray structure of the complete NS3 protein[9], a group from the University of Uppsala (Uppsala, Sweden) set out to see if the helicase/NTPase domain influenced inhibitor binding[10]. The X-ray structure shows the protease substrate-binding domain towards the helicase/NTPase domain, suggesting a binding-pocket composed of both domains. The SAR of peptide-based inhibitors was found to be affected by the presence of the helicase domain. In particular, binding to protein (K_i) was less sensitive to the peptide length of the inhibitor for the complete NS3 than for the isolated protease domain. For example, clipping three of the N-terminal



(vi)

amino acids from peptide **(vi)** [K_i = 0.041 and 0.025 μM (Ref. 11) against full-length and truncated NS3, respectively] yielded a tripeptide inhibitor, **(vii)** (K_i = 4.1 and 230 μM against full-length and truncated NS3, respectively), which was more active against the full-length enzyme than the truncated enzyme. These results suggest that the full-length peptide is a better target with which to screen potential inhibitors against.



(vii)

8 Walker, M.A. (1999) Hepatitis C virus: an overview of current approaches and progress. *Drug Discov. Today* 4, 518–529

9 Yao, N. *et al.* (1999) Molecular views of viral polyprotein processing revealed by the crystal structure of the hepatitis C virus bifunctional protease-helicase. *Structure* 7, 1353–1363

10 Johansson, A. *et al.* (2001) Inhibition of hepatitis C virus NS3 protease activity by product-based peptides is dependent on helicase domain. *Bioorg. Med. Chem Lett.* 11, 203–206

11 Cicero, D.O. *et al.* (1999) Structural characterization of the interactions of optimized product inhibitors with the N-terminal proteinase domain of the hepatitis C virus (HCV) NS3 protein by NMR and modelling studies. *J. Mol. Biol.* 289, 385–396

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Gene therapy

DNA dumbbells: decoys for gene-regulatory proteins

The relative simplicity of synthesis, together with high affinity and specificity for a variety of targets, indicates that oligonucleotide-based therapeutics could be of considerable interest as an approach to rational drug design. Regulation of gene expression by targeting DNA and RNA with complementary single-stranded or folded (catalytic) oligonucleotides forms the basis of well-known antisense, antigene and ribozyme technologies in gene therapy[1–3]. Recently, the first antisense drug, phosphorothioate oligonucleotide fomivirsen, gained approval in the USA and Europe for the treatment of cytomegalovirus infections, earning the Galenus Prize. Furthermore, several anti-HIV hammerhead ribozymes are now in clinical trials.

There is another, less-studied gene-therapeutic strategy termed the 'sense approach', which is based on the targeting of another participant of the genetic machinery, regulatory proteins, with double-stranded oligonucleotides[4]. It is assumed that oligodeoxynucleotide duplexes that carry specific sites for binding with transcription factors might act as decoys at micromolar to nanomolar concentrations[4,5]. Hence, DNA duplexes closed at both termini by stretches of single-stranded nucleic acids or synthetic linkers are of substantial interest[5–11]. These nucleic acid constructs are known as DNA dumbbells because of their similarity in shape with a piece of sports equipment. DNA dumbbells have been considered to be promising oligonucleotide therapeutics mainly because of their increased nuclease resistance and the fact that they more readily enter cells than common DNA duplexes with open termini do[5].

Two general strategies for the preparation of DNA dumbbells have been elaborated. Biochemical syntheses were described on the basis of enzymatic ligation of either a pair of hairpin-like oligonucleotides with short dangling sticky ends[6] or a single, self-complementary, circularizable oligonucleotide[7]. Both these approaches are effective for the assembly of DNA dumbbells with 8–25 bp duplex stems.

However, they experience problems beyond this size range. The upper limit is caused by incorrect annealing of longer self-circularizable and hairpin-like oligonucleotides into non-circularized DNA duplexes, which result in decreased yield of large DNA dumbbells. The lower limit is imposed by steric and conformational constraints in small DNA dumbbells that compromise the efficiency of DNA ligase.

More recently, purely chemical syntheses have been developed[7,8]. Although both enzymatic and chemical approaches for DNA dumbbell assembly are robust, the latter might be preferred for large-scale production because it is faster and less costly[7]. Importantly, chemical syntheses enable DNA dumbbells to be designed that carry chemically reactive groups for covalent affinity modification (crosslinking) of DNA-recognizing proteins[8], as well as to produce DNA dumbbells with non-nucleotide loops, which modulate their lipophilicity and enhance biostability[9]. Using chemical syntheses, researchers have also been able to overcome the problems associated with producing very small DNA dumbbells intrinsic to biochemical approaches, and have yielded DNA dumbbells with duplex stems as short as 3 bp.

However, until recently, no developments have been reported to enlarge the size of DNA dumbbells. Larger DNA dumbbells could have significant advantages as evidently they might carry longer DNA sequences of biological relevance and hence, could be used in gene therapy for intracellular delivery of larger chunks of nuclease-resistant foreign DNA to more effectively regulate gene expression. Recently, Kuhn *et al.* reported a new biosynthetic approach for the high-yield and high-purity preparation of a record-length DNA dumbbell featuring a duplex stem of 94 bp (Ref. 10). The protocol involved enzymatic ligation between a pair of hairpin-like oligonucleotides with long dangling sticky ends followed by multiple biotinylation via nick-translation and/or

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