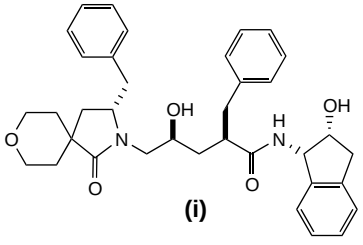
New Inhibitors of HIV-Protease

The addition of inhibitors that target the aspartyl protease expressed by HIV to existing therapies to treat infection has greatly enhanced the treatment of AIDS. However, viral resistance, side effects and the difficult dosing regimens that are associated with these drugs have hampered the effectiveness of this therapy, necessitating the continual search for inhibitors with improved anti-viral activity and pharmacological profiles. Recently, two novel but contrasting approaches have emerged.

In the first study, a unique P2/P1 scaffold (Schecter and Berger nomenclature [1]) was designed by hybridizing two established P2/P1 elements, namely, spirocyclic and pyrrolidone templates [2]. An example of the resulting compound is **i,** which was found to be a modest inhibitor of HIV-protease (K_i = 23 nM). This provides an interesting lead but further optimization would be required to improve the potency of the series. The second approach differs from most HIV-protease programmes in that a peptide scaffold is exploited as an inhibitor [3]. A tetrahydrofuranyl glycine (Tfg) residue at the P2 position of a 9-amino acid protease substrate was found to yield a non-cleavable peptide. These peptides (**ii** and **iii**) inhibited aspartyl protease by 82 and 27%, respectively, at 50 nM, but they do not appear drug-like and no data regarding their *in vivo* behaviour has indicated otherwise. However, a simple modification in peptide sequence can convert a substrate into an inhibitor of the parent enzyme. This could also be used as a tool in the design of inhibitors of proteases other than HIV-protease.



(i)

H-Ser-Gln-Tfg-Tyr-Pro-Ile-Val-OH

(ii)

H-Lys-Ala-Arg-Tfg-Tyr-Nph-Glu-Ala-Nle-NH2

(iii)

(Nph = p.nitrophenylalanine)

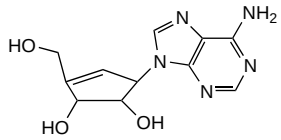
1 Schechter, I. and Berger, A. (1967) On the size of the active site in proteases. I. Papain. *Biochem. Biophys. Res. Commun.* 27, 157–162

2 Kazmierski, W.M. *et al.* (2002) Novel spirocyclic pyrrolidones as P2/P1 mimetics in potent inhibitors of HIV-1 protease. *Bioorg. Med. Chem. Lett.* 12, 3431–3433

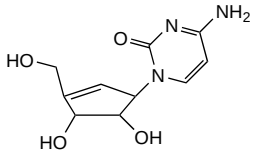
3 Rajesh, S. *et al.* (2002) An expedient synthesis of Nα-protected-L-tetrahydrofuranylglycine and its application in the synthesis of novel substrate based inhibitors of HIV-1 protease. *Bioorg. Med. Chem. Lett.* 12, 3615–3617

Nucleoside analogues active against the smallpox virus

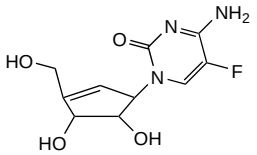
Thanks to an eradication programme initiated by the World Health Organiztion, smallpox infection has been successfully eliminated from the human population, the only known host for the virus. Despite this success, there are concerns that the virus could be used as a biological weapon, and this has prompted a search for antiviral agents that could be used in the event of an attack. Researchers at the University of Georgia and the US Army Medical Research Institute of Infectious Diseases (MD) have identified a number of nucleoside analogues that appear to be potent inhibitors of the smallpox virus in cell culture [4]. The agents consist of D-cyclopentenyl ring attached to nucleoside bases, such as adenine, cytosine and 5-F-cytosine. EC_{50} values for compounds **iv**, **v** and **vi** were 0.03-1.73 μg mL^{-1} in smallpox-infected MK2 and vero cells and the corresponding cytotoxicities were low-moderate (CC_{50} = 3 to >100 μg mL^{-1}). This favorable combination of antiviral activity and limited cytotoxcity suggests that these compounds will be useful leads for further developments in this area.



(iv)



(v)



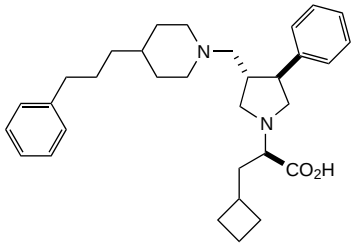
(vi)

4 Chu, C.K. *et al.* (2003) Antiviral activity of cyclopentenyl nucleosides against orthopox viruses (smallpox, monkeypox and cowpox). *Bioorg. Med. Chem. Lett.* 13, 9–12

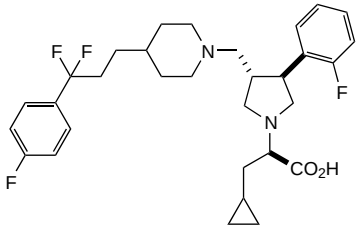
CCR5 Antagonists as inhibitors of HIV infection

HIV entry into cells is mediated by binding to CD4 and CCR5, therefore the inhibition of this process is a target for new antiviral agents. 1,3,4-Trisubstituted pyrrolidines such as compound **vii** have been revealed as CCR5 antagonists, following the assessment of their ability to inhibit [^{125}I]-labelled MIP 1α binding to the receptor expressed on CHO cell membranes (IC_{50} = 0.1 nM) [5]. This translates into potent antiviral activity against HIV in cell culture with an IC_{90} value of 1.2 nM and an IC_{95} value of < 8 nM in a single cycle and 7-day antiviral assay, respectively. In addition to its favorable antiviral activity, compound **vii** also displays acceptable exposure in the rat, having a plasma clearance (CL_P) of 26.5 mL min kg^{-1}, a half life ($t_{1/2}$) of 3.0 h and an oral bioavailability (%F) of 29. Further modifications to this template have yielded compounds with improved binding to CCR5 and prolonged *in vivo* exposure, as presented in a recent publication from Merck (www.merck.com) [6].

For example, compound **viii** has an IC_{50} value against MIP 1α of 0.06 nM, and is able to inhibit HIV infection in cell culture with an IC_{90} value of 0.4 nM and an IC_{95} value of 12 nM in the single cycle and 7-day anti-viral assays. The improved binding to CCR5 is consistent with a previous observation that electron-withdrawing substituents enhance receptor affinity. The gem-difluoro substitution was introduced to block metabolism next to the phenyl group, which might explain the lower clearance and longer half-life of the compound in the rat (CL_P = 14 mL min kg^{-1}, $t_{1/2}$ = 7.5 ha and %F = 39).



(vii)



(viii)

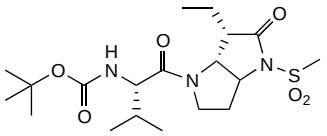
5 Hale, J.J. *et al.* (2001) 1,3,4-Trisubstituted pyrrolidine CCR5 receptor antagonists. Part 2: lead optimization affording selective, orally bioavailable compounds with potent Anti-HIV activity. *Bioorg. Med. Chem. Lett.* 11, 2741–2745

6 Lynch, C.L. *et al.* (2003) 1,3,4-Trisubsitituted pyrrolidine CCR5 receptor antagonists: Modifications of the arylpropylpiperidine side chains. *Bioorg. Med. Chem. Lett.* 13, 119–123

Inhibitors of HCV protease

The NS3 serine protease expressed by the hepatitis C virus (HCV) has surfaced as a prominent target for the development of potential therapeutic agents to treat infection by this virus. Much success has been achieved by using competitive peptide-like inhibitors that resemble the structure of the cleavage products of the enzyme. Although these agents have proven to be highly potent in cell culture assays, their advancement in to the clinic has been delayed because they are high molecular weight (mw >500) peptide-like compounds, which, therefore, present a number of technical challenges. Less success has been achieved with small non-peptide inhibitors and it appears to be much more difficult to design inhibitors of the NS3 protease using known protease inhibiting scaffolds because the enzyme offers few sites for binding. For this reason, a recent report from GlaxoSmithKline (www.gsk.com), describing the identification of a mechanism-based inhibitor, is encouraging and suggests that identification of a non-peptide NS3 inhibitor, derived from known serine-protease inhibitor chemotypes, is possible [7].

The pyrrolidine-5,5-trans-lactam template has been used in the design of serine-protease inhibitors directed against thrombin, elastase and the serine-protease expressed by human cytomegalovirus. The trans-lactam group of this chemotype is activated towards acylation of the active site serine hydroxyl group, resulting in time-dependent, mechanism-based inhibition of the respective protease. Thus, compound **ix,** which displays modest activity (IC_{50} = 30 μM), was designed as a prototypical inhibitor of NS3, based on the following considerations: the group adjacent to the lactam carbonyl probably projects into the S1 pocket of the enzyme, while the distal- and amide-nitrogen atoms provide access to S3-S4 and S1, respectively. Modification of the ethyl substituent to the corresponding spirocyclic cyclobutyl, and replacement of the methylsulfonyl group attached to the lactam nitrogen with a cyclopropylacyl, as in compound **x** (IC_{50} = 0.51 μM), resulted in increased potency and plasma stability (turnover in human plasma after 4 hours = 28%) [8]. The compound was also effective in cell culture, in inhibiting an HCV-replicon (IC_{50} (HCV-replicon) = 5.7 μM), which serves as a model system to evaluate antiviral activity. The potency of these compounds is unlikely to be sufficient for them to be considered as candidates for clinical trials, but they do represent a significant milestone in the design of non-peptide-based inhibitors.



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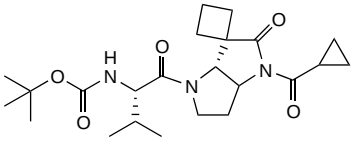

(x)

7 Slater, M. J. *et al.* (2002) Design and synthesis of ethyl pyrrolidine-5,5-trans-lactams as inhibitors of hepatitis C virus NS3/4A protease. *Bioorg. Med. Chem. Lett.* 12, 3359–3362

8 Andrews, D. M. (2002) Pyrrolidine-5,5-transl-lactams. 1. Synthesis and incorporation into inhibitors of Hepatitis C virus NS3/4A protease. *Org. Lett.* 4, 4475-4478

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