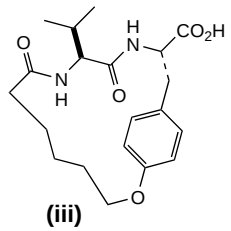
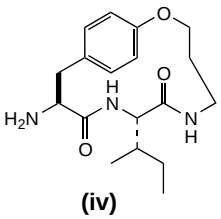
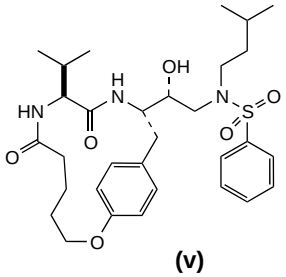


(iii)



(iv)



(v)

designs for proteases have been concerned with derivatizing the peptide substrates using a variety of mechanism-, analogue- and structure-based approaches to produce more pharmacologically acceptable non-peptide molecules that are capable of maximizing enzyme–inhibitor interactions. One problem with optimizing enzyme inhibitors in this way is the highly cooperative ('induced fit') nature of inhibitor–enzyme binding. The resultant changes in folding of adjacent pockets cause 'knock-on' effects, which are collectively termed 'cooperativity'. Thus, it is difficult to predict accurately the effects of optimizing one (flexible) inhibitor region independently from another remote site. In an attempt to address this, Reid *et al*. [2] investigated the use of constrained cyclic tripeptide mimics (such as **iii** and **iv**) as templates to order their immediate enzyme environment and, therefore, dampen the induced fit that results from changes to appended groups. The macrocycles **iii** and **iv** were designed to mimic (structurally and functionally) the P1–P3 or P1′–P3′ tripeptide segments that act as HIV-1 protease inhibitors. One advantage that these macrocycles have over acyclic peptides is that they are preorganized in an extended (protease-binding) conformation in water before binding to a protease; this preorganization confers a significant entropy advantage for binding. In addition, the macrocycles are more resistant to proteolytic degradation, thus increasing bioavailability. The use of constrained macrocyclic templates that are equivalent to tripeptides could enable the regioselective optimization of protease and/or enzyme inhibitors through focused combinatorial libraries, an approach illustrated by Reid *et al.* [2] in the design of HIV-1 protease inhibitors using macrocyclic components such as **iii** and **iv**.

The macrocycles **iii** and **iv** were used as template structures for the synthesis of a small solution-phase library. Next, N-terminal and C-terminal macrocycles were screened for enzyme activity against HIV-1 protease (SF2 isolate) using a fluorimetric assay. Of the compounds screened, one of the most potent was **v**, having a K_i of 0.4 nM against synthetic HIV-1 protease. This work has demonstrated that N-terminal and C-terminal macrocycles are excellent structural mimics for the respective tripeptide fragments of acyclic peptide inhibitors. For those proteases for which crystal structures are available, this templating approach could help the exploitation of structure-based design methods.

2 Reid, R.C. *et al.* (2004) Countering cooperative effects in protease inhibitors using constrained beta-strand-mimicking templates in focused combinatorial libraries. *J. Med. Chem.* 47, 1641–1651

Paul Edwards
paul.edwards@santhera.com

CANCER BIOLOGY

Deceiving the enemy

Inappropriate activation of the transcription factor, STAT3, has been described in a number of solid tumours, including squamous cell carcinoma of the head and neck (SCCHN). It is thought that STAT3 contributes to malignancy by increasing the expression of the anti-apoptotic gene, Bcl-xL. STAT3 therefore represents an attractive candidate for therapeutic intervention.

In a recent study, a 'decoy' approach was adopted to inhibit STAT3 activity. Double-stranded DNA oligonucleotides corresponding to the STAT3-binding element were used to mimic the bona fide site found within the promoters of STAT3-responsive genes. At high concentrations, the decoy sequesters STAT3 and impairs binding to the genomic promoter, modulating target gene expression. Previous work by the same laboratory had shown that this tactic was successful in cultured SCCHN cells. However, there was a need to evaluate the decoy in an animal model.

Athymic nude mice bearing SCCHN xenografts were injected with the decoy. STAT3 DNA-binding activity was impaired, expression of Bcl-xL, VEGF, cyclin D1 and PCNA were reduced, and the apoptotic rate increased. This was associated with reduced tumour volumes. However, a mutant decoy had no effect. Moreover, the STAT3 decoy did not affect activity of the related protein, STAT5, suggesting that inhibition is relatively specific. Of particular interest was the observation that combining the STAT3 decoy with the chemotherapeutic agent, cisplatin, enhanced the tumour-inhibitory effects.

Although further trials are required to determine the efficacy of the STAT3 decoy fully for treating SCCHN, the results of this study are encouraging. It will also be interesting to see whether other transcription factors can be targeted by this approach.

1 Xi, S. *et al.* (2004) *In vivo* antitumor efficacy of STAT3 blockade using a transcription factor decoy approach: implications for cancer therapy. *Oncogene* doi: [E-pub. ahead of print;]

Victoria Heath
vjh2r@udcf.gla.ac.uk

MICROBIOLOGY

Autophagy gives macrophages the upper hand against *M. tuberculosis*

Autophagy (the ingestion of cytoplasmic macromolecules and organelles) and phagocytosis (the ingestion of exogenous particles or microorganisms) both involve membrane-bound compartments that mature into lysosomes. Phagocytosis is triggered by the ingestion of foreign material while autophagy can be stimulated by starvation, inhibition of the TOR (target of rapamycin) kinase, and modulation of hormonal levels. Although these processes resemble each other and both are dependent on phosphotidylinositol-3-phosphate signaling pathways, they also exhibit distinct surface markers.

Mycobacterium tuberculosis' preeminence as a pathogen depends in part on its ability to survive within macrophages. Once inside a phagosome, *M. tuberculosis* blocks phagosome-lysosome fusion, thereby avoiding destruction.

1359-6446/04/$ – see front matter ©2005 Elsevier Ltd. All rights reserved. PII: S1359-6446(04)03327-6

Gutierrez *et al*. now demonstrate that induction of autophagy can enable macrophages to bypass *M. tuberculosis*-mediated blockade of phagosomal maturation [2]. When a murine macrophage line infected with the BCG strain of *M. tuberculosis* was treated with rapamycin or exposed to starvation conditions, phagosomes containing BCG became acidified and acquired lysosomal enzymes such as cathepsin D. The phagosomes also acquired protein LC3, a marker normally restricted to the autophagy pathway. Stimulation of autophagy in either murine or human macrophages resulted in increased killing of both BCG and virulent *M. tuberculosis*. This enhanced bactericidal activity was blocked by addition of 3-methyladenine, an inhibitor of hVSP34 (a phosphotidylinositol-3-kinase required for maturation of autophagosomes).

Based on these results, Gutierrez *et al*. then showed that treatment of macrophages with interferon-γ also induces autophagy (again inhibited by 3-methyladenine) and causes the translocation of LC3 to phagosomes harboring mycobacteria. These findings suggest that under physiologic conditions macrophages can overcome the ability of *M. tuberculosis* to inhibit phagosome–lysosome fusion by diverting phagosomes into the autophagocytic pathway. Further investigation of this mechanism might make possible the development of novel agents for controlling tuberculosis.

2 Gutierrez, M.G. *et al.* (2004) Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. *Cell*, 119, 753–766

Eric D. Spitzer
espitzer@notes.cc.sunysb.edu

We regret that we omitted the author's name at the end of his last monitor article about Pseudomonas and Candida (DDT 2004;9:1082–1083).