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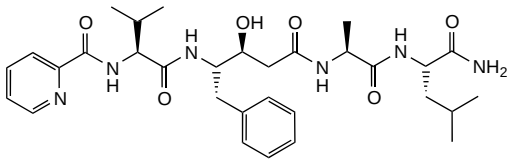
monitor

COMBINATORIAL CHEMISTRY

Inhibitors of plasmepsin I and plasmepsin II

It is estimated that malaria costs as many as two million people their lives each year, and it is thought that more than 40% of the global population could be at risk from infection. Of all the species of mosquito known to man, only the tropical dwelling Anopheles sp. is capable of spreading malaria. There are four major species of malaria parasite and, of these, *Plasmodium falciparum* is responsible for more than 95% of all malaria-related morbidity and mortality. However, *P. falciparum* is becoming resistant to existing therapies; some strains have been reported to be resistant to all known antimalarial therapies. This is clearly a potential health problem of global significance and it has precipitated the need to investigate novel strategies for the development of new and effective treatments for the management of this disease.

In the erythrocytic stage of the life cycle of *P. falciparum*, haemoglobin represents a major source of the nutrients required for growth. During this phase, the parasite can consume up to 80% of the haemoglobin of the host. The degradation of haemoglobin occurs in acidic food vacuoles within the parasite, and disruption of these vacuoles has been the strategic target for many of the antimalarial drugs of today. The food vacuole contains aspartic-, cysteine- and metallo-proteases, all of which are considered to be involved in haemoglobin degradation. The food vacuole contains four aspartic-proteases, namely plasmepsin I, II and IV and histo-aspartic-protease. Inhibitors of the parasitic cysteine-proteases falcipain-1, -2 and -3, as well as plasmepsin I and II, have all been studied in detail and have shown efficacy in cell and animal models of malaria, which strongly suggests that inhibition of these enzymes could provide a reasonable and achievable strategy for the discovery of novel, effective antimalarials. Johannson *et al.* [1] examined the optimization of the phenyl statine core of inhibitor **i**, which served as a useful starting point for the production of novel and improved inhibitors of plasmepsin I and II. Synthesis of several small solution-phase and solid-phase libraries was undertaken to provide SAR and to optimize for potency. Compounds were then screened in enzyme inhibition assays, measuring inhibition of



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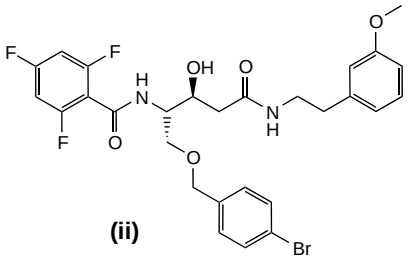

(ii)

plasmepsin I and pro-plasmepsin II. Active compounds were screened further using a *P. falciparum* growth inhibition assay. One of the most potent compounds was **ii**, which had K_i values against plasmepsin I and II of 619 nM and 390 nM, respectively. Furthermore, **ii** inhibits parasite growth in red blood cells, with 29% inhibition at a concentration of 5 μM. Accordingly, this research has generated potent inhibitors of plasmepsin I and II and further investigations in this area are warranted.

1 Johansson, P-O. *et al.* (2004) Design and synthesis of potent inhibitors of the malaria aspartyl proteases plasmepsin I and II. Use of solid-phase synthesis to explore novel statine motifs. *J. Med. Chem.* 47, 3353–3366

Protease inhibitors

Regulation of physiological and pathophysiological processes by proteases has dictated that they assume a significant role as targets for the development of new drugs. Advances in the technology for the characterization of protein structure have enabled the solution of the crystal structure of a large number of proteases. Such approaches have revealed that proteases universally recognize the β-strand conformation of substrates and inhibitors. However, the majority of reported protease inhibitors are conformationally flexible. To date, few attempts have been made to create more constrained β-strand mimetics. Rather, most rational

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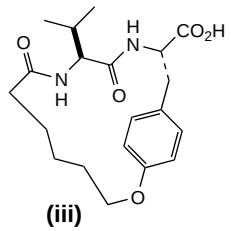

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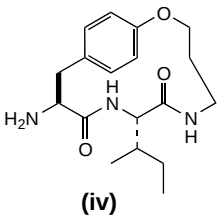

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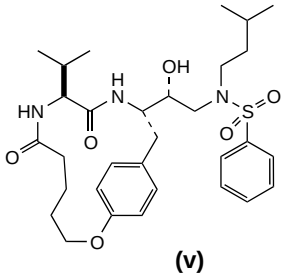

(v)

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

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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;]

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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.

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