Monitor

Monitor **provides an insight into the latest developments in the pharmaceutical and biotechnology industries.** ***Chemistry*** **examines and summarises recent presentations and publications in medicinal chemistry in the form of expert overviews of their biological and chemical significance, while** ***Profiles*** **provides commentaries on promising lines of research, new molecular targets and technologies.** ***Biology*** **reports on new significant breakthroughs in the field of biology and their relevance to drug discovery.** ***Business*** **reports on the latest patents and collaborations, and** ***People*** **provides information on the most recent personnel changes within the drug discovery industry.**

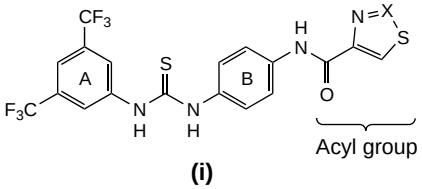
Chemistry

Medicinal chemistry

Thiourea-based potent and selective HCMV inhibitors

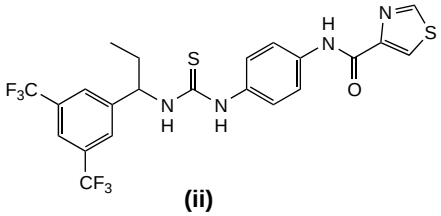
Although infection with human cytomegalovirus (HCMV) is usually asymptomatic in healthy adults and children, this agent is a leading cause of birth defects and can lead to severe, often life-threatening, diseases in those that are immunocompromised (e.g. HIV-positive individuals) or immunosuppressed (e.g. those having undergone organ and/or bone marrow transplant). Currently, five compounds have been licensed for the prophylaxis and treatment of HMCV infection; ganciclovir, foscarnet, cidofovir, valganciclovir and fomivirsen. All these approved HCMV inhibitors, with the exception of fomivirsen, are targeted at viral DNA polymerase. Despite their antiviral potential, the use of these medications is associated with several drawbacks, including dose-limiting bone marrow and kidney toxicity, as well as the emergence of drug resistance. These findings have prompted the search for other HCMV drugs that are selective, safe and target other aspects of the HCMV replication cycle.

Researchers have recently reported the identification and subsequent optimization of a series of thiourea small molecule derivatives as potent and selective inhibitors of HCMV with a novel mechanism of action. The HTS of a proprietary chemical library on HCMV- and human syncytial virus (HSV)-infected cell assays led to the discovery of an anti-HSV drug candidate. Simple modifications of the initial *N*,*N*-bis-arylthiourea derivative, for example, the substitution of an acetyl with a benzoyl group, unexpectedly resulted in the selective inhibition of HCMV replication, which triggered the preparation and assay of a new collection of thiourea analogues [1]. Modifications at the three key groups of the core structure (**i**), ring A, ring B and the *N*-acyl group, were explored. SAR studies from these alterations showed that the anti-CMV activity was attributed to the presence of electron withdrawing groups in ring A and five-membered heterocyclic-substituted acyl groups in ring B. Furthermore, additional substitutions of ring B resulted in significant decreases in activity. Some of the analogues generated, for example, the thiazole and thiadiazole derivatives **ia** and **ib**, respectively, showed low nM inhibitory activity against HCMV replication in cultured human foreskin fibroblast cells.



(i)

ia: X = CH
ib: X = N



(ii)

A comprehensive biological study demonstrated that these compounds act at a point early in the replication cycle, inhibiting glycoprotein B-mediated HCMV virion fusion with the cell plasma membrane and the cell–cell spread of HCMV [2]. However, the *N*,*N*-bis-arylthiourea derivatives were found to be unstable under neutral and acidic conditions, precluding their further development. This issue was elegantly solved through the incorporation of spacers between the thiourea moiety and one of the phenyl rings (ring A of **i**), thus preventing the hydrolysis of the thiourea moiety. In addition to the increased hydrolytic stability, the new spacer-containing thioureas were significantly more potent than the preliminary bis-arylthioureas without loss of selectivity [3]. The prototype compound in this new series, **ii**, had an IC_{50} value of 0.2 nM against HCMV and a selectivity index of at least 150,000. Although no clinical candidates have as yet resulted from this series (because of poor bioavailability), there are two key reasons that support the further investigation of **ii** and its congeners: (i) these compounds inhibit one of the earliest steps in the infective cycle, which will avoid potential cytopathological and immunopathological effects that could be triggered by viral

1359-6446/04/$ – see front matter PII: S1359-6446(04)03264-7

structural proteins involved in early phases of the replication cycle; and (ii) the unique mechanism of action of these compounds is complementary to that of other anti-HCMV agents that do not target DNA replication, such as the indolizine-1-carboxamide MCV423, which acts at a stage of the replication cycle that precedes the DNA polymerase step, and the L-ribose benzimidazole derivative 1263W94 (marivavir), which appears to be targeted to the UL97 protein kinase (currently undergoing clinical trials) [4].

1 Bloom, J.D . *et al.* (2003) Thiourea inhibitors of herpes viruses. Part 1: bis-(aryl)thiourea inhibitors of CMV. *Bioorg. Med. Chem. Lett.* 13, 2929–2932

2 Jones, T.R. *et al.* (2004) Specific inhibition of human cytomegalovirus glycoprotein B-mediated fusion by a novel thiourea small molecule. *J. Virol.* 78, 1289–1300

3 Bloom, J.D. *et al.* (2004) Thiourea inhibitors of herpes viruses. Part 2: *N*-Benzyl-*N*′-arylthiourea inhibitors of CMV. *Bioorg. Med. Chem. Lett.* 14, 3401–3406

4 De Clercq, E. (2003) New inhibitors of human cytomegalovirus (HCMV) on the horizon. *J. Antimicrob. Chemother.* 51, 1079–1083

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Biology

Microbiology

Caged and GRABbed: *Streptococcus pyogenes* protease degrades antimicrobial peptide

Regulation of proteolysis is crucial in maintaining balance in the human body and is accomplished by a complex array of proteases and protease inhibitors. Many infections are characterized by uncontrolled proteolysis leading to tissue damage and activation or inhibition of proteins involved in the immune defense and coagulation. The human pathogen *Streptococcus pyogenes* secretes several proteases that degrade antibodies, complement and coagulation factors. The streptococcal cysteine proteinase, SpeB, degrades several such molecules including the antimicrobial peptide LL-37, a component of the innate immune defense. In addition to proteases, *S. pyogenes* expresses a surface protein, GRAB, that binds the human proteinase inhibitor α2-macroglobulin (α2M) and thereby controls proteolysis at the bacterial surface.

Nyberg *et al.* [1] have now shown that SpeB is trapped in the α2M cage forming a SpeB-α2M complex with no activity on larger proteins. On the contrary, SpeB-α2M allows the small peptide LL-37 to enter the cage and even more efficiently inactivates LL-37 than SpeB alone. By comparing wild type bacteria and a mutant lacking GRAB expression, it was shown that SpeB-α2M complexes bound to bacteria protects them from killing by LL-37. This protection is dependent on all three active components, SpeB, α2M, and GRAB.

This study elegantly demonstrates an intricate process where S. pyogenes through the surface protein GRAB acquires the proteinase inhibitor α2M to trap its own proteinase SpeB at the bacterial surface. This allows the bacteria to direct the proteolysis towards small molecules, such as antimicrobial peptides, and concentrates the activity to the bacterial surface where it is needed the most.

1 Nyberg, P. *et al.* (2004) α2-macroglobulin-proteinase complexes protect *Streptococcus pyogenes* from killing by the antimicrobial peptide LL-37. *J. Biol. Chem.* doi:10.1074/jbc.C400485200 (EPub. ahead of print; http://www.jbc.org)

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Pseudomonas affects *Candida* differentiation

The ability of *Candida albicans* to reversibly switch between yeast and hyphal morphologies plays an essential role in the pathogenesis of infections caused by this opportunistic pathogen. Earlier work has shown that co-cultivation of *C. albicans* with the bacterial pathogen *Pseudomonas aeruginosa* results in killing of hyphae, whereas yeast are spared. A current study by the same group now demonstrates that one of the main quorum-sensing molecules secreted by *P. aeruginosa* can inhibit the yeast-to-hypha transition in *C. albicans*. [2].

To screen for interactions between these two organisms, Hogan *et al.* developed a plate assay utilizing a *HWP1-lacZ* derivative of *C. albicans*, hyphae-inducing agar, and the chromogenic substrate X-Gal. As yeast convert to hyphae they express *lacZ* (*HWP1* is only expressed in hyphae) and turn the agar blue. When wild-type *P. aeruginosa* was grown on these plates, the bacterial colonies were surrounded by white halos that contained only yeast forms. Further studies with bacterial mutants and purified compounds revealed that this effect was due to secretion of the quorum-sensing molecule, 3-oxo-dodecanoyl-homoserine lactone (3OC12HSL).

RT-PCR analysis confirmed that this compound was capable of suppressing hypha-specific genes and inducing yeast-specific genes. Other C12 compounds, such as dodecanoyl-HSL and dodecanol, also inhibited filamentation whereas the corresponding C10 and C14 derivatives had no effect. Farnesol, which also has a 12 carbon backbone, has been previously shown to be a quorum sensing molecule secreted by *C. albicans* that suppresses the yeast to hyphae transition. It is not known how farnesol and 3OC12HSL inhibit filamentation or whether they act at the same site.

The ability of *P. aeruginosa* to affect *C. albicans* differentiation *in vitro* could provide insight into the growth and survival of these organisms within polymicrobial communities in colonized patients.

PII: S1359-6446(04)03193-9