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.**

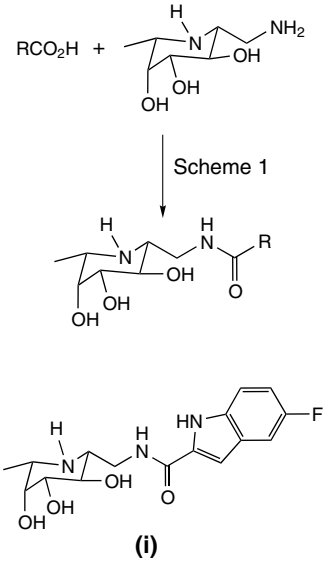
Chemistry

Combinatorial Chemistry

α-Fucosidase inhibitors

Polyhydroxypiperidines and pyrrolidines (known also as iminocyclitols or aza-sugars) have attracted attention due to their inhibitory activity against various glycosidases. The glucose-type iminocyclitol deoxynojirimycin has been used for the treatment of non-insulin dependent diabetes. Recent studies with deoxynojirimycin and its derivatives indicate they are effective against hepatitis B and C [1–3], as well as glycosphingolipid storage disorders such as Gaucher disease [4].

The efficacy of iminocyclitols is attributed to their mimicry of the transition state of enzymatic glycosidic cleavage. Of the members of the glycosidase family, α-fucosidase is involved in the hydrolytic degradation of numerous fucose-containing glycoconjugates. The existence of this enzyme is associated with several essential functions: the abnormal accumulation of fuco-conjugates, resulting from the absence or deficiency of α-fucosidase, which leads to the genetic neurovisceral storage disease fucosidosis. An aberrant distribution of intracellular and extracellular α-fucosidase is also found in cystic fibrosis, for example. Although the physiological functions of α-fucosidase are not completely understood, potent fucosidase inhibitors may be used as probes for the study of



(i)

fucosidases with regard to their functions, and also for the development of potential therapeutic agents.

To facilitate the discovery of new glycosidase inhibitors, Wong and Lin and coworkers have developed a new method for rapid derivatization of an iminocyclitol core designed for a specific glycosidase family; in particular, fuconojirimycin derivatives for fucosidases. In this methodology, the compounds are synthesized without protecting group manipulation and under such conditions that the product could be used directly for screening *in situ* without isolation [5].

A solution phase library of 60 fuconojirimycin derivatives was synthesized as singletons according to general scheme 1. The α-fucosidase from bovine kidney was used for inhibition studies. Several active compounds were identified. One of the most potent was compound **i**, which possessed a K_i of 0.6 nM. Selectivity against related glycosidases studied was high. This work has produced the most potent and selective inhibitors reported to date against the glycosyltransfer enzyme α-fucosidase and warrants further investigation.

1 Block, T.M. *et al.* (1994) Secretion of human hepatitis B virus is inhibited by the imino sugar N-butyldeoxynojirimycin. *Proc. Natl. Acad. Sci. U. S. A.* 91, 2235–2239
2 Mehta, A. et al. (2001) Inhibition of hepatitis B virus DNA replication by imino sugars without the inhibition of the DNA polymerase: therapeutic implications. *Hepatology* 33, 1488–1495
3 Durantel, D. et al. (2001) Study of the mechanism of antiviral action of iminosugar derivatives against bovine viral diarrhea virus. *J. Virol.* 75, 8987–8998
4 Cox, T. *et al.* (2000) Novel oral treatment of Gaucher's disease with N-butyldeoxynojirimycin (OGT 918) to decrease substrate biosynthesis. *Lancet* 355, 1481–1485
5 Wu, C-Y. *et al.* (2003) Rapid diversity-orientated synthesis in microtiter plates for in situ screening: discovery of potent and selective α-fucosidase inhibitors. *Angew. Chem., Int. Ed. Engl.* 42, 4661–4664

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Human betaine inhibitors

The translation of sequencing data into an understanding of the function of proteins in cells, tissues or whole organisms is the key challenge for functional genomics and proteomics. Small ligands that are able to specifically interact with proteins can be effective tools in the search for proteome function. Classically, new ligands for proteins have been identified by SAR studies, molecular modeling or combinatorial chemistry techniques.

However, these approaches generally use only one protein target. Given the high number of proteins in mammalian organisms, HTS procedures have been developed to handle this task. A potential drawback with such methods is that the full range of proteins that the chosen ligand could interact with are not discovered if the screening is performed with only one or a few proteins. This lack of information regarding how many proteins a given ligand can interact with precludes our ability to completely understand the full spectrum of effects that a ligand might have in a complex medium such as a living cell. Approaches that study the effects of ligands in whole cells are becoming important. Screening libraries using biosensor chips or arrays grafted with proteins enables the real-time recording of ligand–protein interactions. Subsequent elution of these complexes can be used for protein identification by MS.

A method to discover novel protein–ligand interactions has been developed based on affinity capture principles coupled to combinatorial chemistry [6]. Affinity columns were prepared containing 361 different phosphinic peptides, which were used to isolate all interacting proteins from crude rat liver homogenates. By applying a deconvolution process, the most specific ligand was identified within the phosphinate peptide library that had the highest affinity towards one newly discovered protein target, betaine:homocysteine S-methyltransferase (BHMT). The phosphinic pseudopeptides, which served as immobilized ligands for the isolation of rat BHMT, were then tested for their ability to inhibit human recombinant BHMT in solution. The most potent inhibitor also behaved as a selective ligand for the affinity purification of BHMT from a complex media. Further optimization of this active identified compound **ii** as a potent BHMT inhibitor that possessed an IC_{50} value of about 1μM.

Val-Phe-ψ[PO_2^--CH_2]Leu-His-NH_2

(ii)

This work has demonstrated the successful application of a new and simple method for the discovery of new protein targets for artificial ligands of interest. This methodology, in combination with 2D electrophoresis and MALDI-MS holds promise as an additional method for the discovery of new specific protein-ligand interactions.

6 Collinsova, M. *et al.* (2003) Combining combinatorial chemistry and affinity chromatography: highly selective inhibitors of human betaine: homocysteine S-methyltransferase. *Chem. Biol.*10, 113–122

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Biology

Microbiology

Fibronectin-binding in *Streptococcus pyogenes*: proteolytic regulation and virulence fine-tuning

Surface proteins that bind the extracellular matrix and plasma protein fibronectin (Fn) are widespread among bacterial pathogens. In the human pathogen *Streptococcus pyogenes*, Fn-binding is important for cellular invasion and has been suggested to contribute to pathogenesis and virulence.

Nyberg *et al.* now report that the streptococcal cysteine proteinase, SpeB, modulates Fn-mediated cellular internalization of *S. pyogenes* [1]. SpeB degrades the Fn-binding protein F1 (PrtF1) when anchored to the bacterial surface in a plasma environment. In contrast to IgG and fibrinogen, which protect their bacterial surface receptors (M proteins), Fn does not protect protein F1 from SpeB. Instead Fn is degraded by SpeB when bound to the streptococcal surface.

Nyberg *et al.* continued by investigating Fn-binding and virulence, using isogenic PrtF1-expressing or non-expressing strains, cellular adhesion and internalization assays, and transgenic mice not producing plasma Fn (pFn) [2]. They showed that adhesion is dependent on the interaction between PrtF1 and cellular Fn (cFn) and pFn, whereas internalization depends on pFn alone. Furthermore, *S. pyogenes* virulence is attenuated in PrtF1-expressing strains but is partly restored in mice not expressing pFn. Thus, PrtF1-mediated Fn-binding is the first described anti-virulence trait in *S. pyogenes* and could be beneficial in establishing a balanced bacterial interaction with the host. This is particularly interesting because most of the highly virulent *S. pyogenes* strains of the M1 serotype do not express PrtF1. Furthermore, the strains that do express PrtF1 can, under certain circumstances, regulate the surface expression by a bacterial protease, SpeB.

Taken together, these two studies clearly contribute to the understanding of how *S. pyogenes* regulates cellular binding, internalization and virulence on a molecular level. Finally, these studies also emphasizes the importance of taking potential anti-virulence interactions, including PrtF, into account when new therapeutic strategies are developed.

1 Nyberg, P. *et al.* (2004) SpeB modulates fibronectin-dependent internalization of *Streptococcus pyogenes* by efficient proteolysis of cell-wall-anchored protein F. *Microbiology* 150, 1559–1569
2 Nyberg, P. *et al.* (2004) Interactions with fibronectin attenuate the virulence of *Streptococcus pyogenes*. *EMBO J.* DOI: 10.1038/sj.emboj.7600214 (E-pub ahead of print; http://embojournal.npgjournals.com).

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