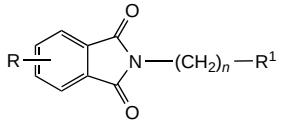
Novel α-glucosidase inhibitors

The enzyme α-glucosidase (EC 3.2.1.20) catalyzes the final step in the digestive processing of carbohydrates. Therefore, its inhibitors have potential in the management of diseases such as diabetes and certain forms of hyperlipoproteinemia, as well as in the treatment of obesity. They are also considered as potential antiviral and anticancer agents. Recently their use in the treatment of AIDS has been suggested.

During studies on a series of tumor necrosis factor-α (TNF-α) production regulators, a Japanese group identified potent agents with a phthalimide skeleton **(vii)**[6]. Some of them showed the expected hypoglycemic activity; this, however, did not correlate with their TNF-α production-regulating activity. They hypothesised that α-glucosidase inhibition could be involved. Therefore, all the compounds were tested in comparison with 1-deoxynojirimycin (dNM), a well known α-glucosidase inhibitor[7]. Some of these compounds, which had a tetrachlorosubstituted phthalimide moiety, displayed activity higher than the model by one or two orders of magnitude. Their IC_{50} values ranged from 0.7–2.6 μM.



(vii)

This new series could represent an innovative approach to the treatment of various diseases as well as to mechanistic studies of α-glucosidase inhibition.

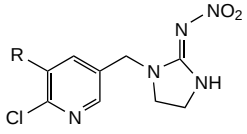
6 Takahashi, H. *et al.* (2000) α-Glucosidase inhibitors with a phthalimide skeleton: structure–activity relationship study. *Chem. Pharm. Bull.* 48, 1494–1499

7 Inouye, S. *et al.* (1968) Structure and synthesis of nojirimycin. *Tetrahedron* 24, 2125–2144

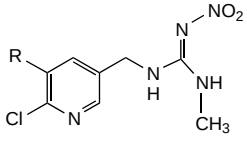
Azidopyridinyl compounds as candidate photoaffinity probes for nicotinic acetylcholine receptors

The most commonly used photoaffinity probes for the structural analysis of nicotinic acetylcholine receptors (nAChRs) include [^{3}H]nicotine, [^{3}H]5-azidonicotine and a 2-azido-5-^{125}iodobenzoyloxyethyl derivative of an imidacloprid analogue[8]. In particular, the nicotine derivatives are selective for mammal nAChRs and the imidacloprid analogues for insect nAChRs. Therefore, photoaffinity ligands that have nanomolar potency on both mammalian and insect nAChRs would be highly desirable.

Based on the consideration that aryl azides are the most popular photoaffinity reagents, Casida and colleagues devised the 5-azido-6-chloropyridin-3-yl substituent as a suitable candidate for such a probe[9]. Compound **(viii)** was synthesized as the azido derivative of imidacloprid **(ix),** and **(x)** as the azido derivative of its open analogue **(xi)**. Their effects were evaluated using rat brain, rat $\alpha_4\beta_2$ integrin, *Myzus* and *Drosophila* membrane preparations. Although the model compounds **(ix)** and **(xi)** have relatively poor affinities in rat brain membranes and in $\alpha_4\beta_2$ preparation, the azido substituent increases the affinity [**(viii)** and **(x)** = 44 nM with brain and **(x)** = 4.4 nM with $\alpha_4\beta_2$ membranes). All the compounds have high affinities for the insect preparations (K_i values = 1–15 nM). Therefore, compounds **(viii)** and **(x)** offer the possibility of using the same photoprobe for mammals and insects when evaluating the structural basis for selectivity.



(viii) R = N_3
(ix) R = H



(x) R = N_3
(xi) R = H

8 Tomizawa, M. *et al.* (1997) [^{125}]Azidinicotinoic photoaffinity labeling of insecticide-binding subunit of *Drosophila* nicotinic acethylcholine receptor. *Neurosci. Lett.* 237, 61–64

9 Kagabu, S. *et al.* (2000) 5-Azidoimidacloprid and an acyclic analogue as candidate photoaffinity probes for mammalian and insect nicotinic acetylcholine receptors. *J. Med. Chem.* 43, 5003–5009

Steven Langston
Millennium Pharmaceuticals
Merryfield Centre, Rosemary Lane
Cambridge, UK CB1 3LQ
tel: +44 (0)1223 722400
e-mail: steve.langston@mpi.com

Daniela Barlocco
University of Milan
Viale Abruzzi, 42
Milano-20131, Italy
tel: +39 02 2950 2223
fax: +39 02 2951 4197
e-mail: daniela.barlocco@unimi.it

Combinatorial chemistry

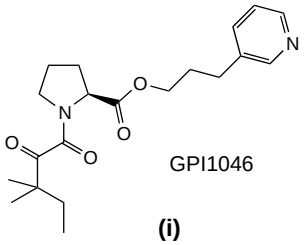
Neuroimmunophilin ligands

Recently, several groups have disclosed novel compounds derived from the peptidylprolyl isomerase (PPIase)-inhibition domains of the immunosuppressive drugs FK506 and rapamycin. These non-immunosuppressive small molecules, with a MW of <500, were found to be potent neurotrophic agents in neurite outgrowth assays. Certain analogues, such as GPI1046 **(i)** have also been shown to be effective in models of Parkinson's and Alzheimer's diseases. Dosing paradigms have shown that these compounds cause both neuroprotective and neuroregenerative effects in models of CNS pathogenicity.

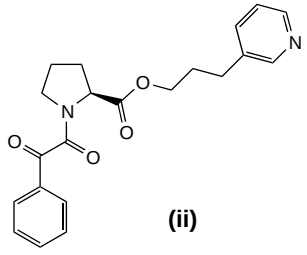
The biological target for the exceptional neurotrophic activity of compounds such as **(i)** is not known, although FKBP-12 is a probable candidate because it is upregulated in models of CNS injury. It has been shown, however, that there is no clear correlation between rotamase inhibition activity and the ED_{50} value for neurite outgrowth. In an effort to produce SARs in one compound series,

1359-6446/01/$ – see front matter PII: S1359-6446(01)01762-7

thereby helping to separate the neuroprotective and neuroregenerative properties of these compounds, a library of compounds targeting neuroimmunophilin was synthesized[1]. A library of 880 individual compounds was synthesized in solution, in which the central amino-acid core present in **(i)** was retained, and the other substituents varied. These compounds were tested in a FKBP-12 SPA (scintillation proximity assays) binding assay. Of those compounds tested, all possessed lower affinity for this protein compared with FK506 and rapamycin; for example, **(ii)**, which displayed a pIC_{50} value of <5. The success of this library protocol could facilitate future endeavours in the search for SARs in this series where the amino-acid core is also varied.



(i)



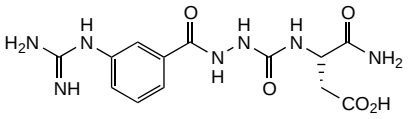
(ii)

1 Rabinowitz, M. *et al.* (2000) Solid-phase/solution-phase combinatorial synthesis of neuroimmunophilin ligands. *Bioorg. Med. Chem. Lett.* 10, 1007–1010

$\alpha_5\beta_3$ Integrin antagonists

The inhibition of the $\alpha_5\beta_3$ integrin receptor is regarded as a promising goal in the therapy of various pathophysiological processes such as tumour-induced angiogenesis, restenosis, osteoporosis and acute renal failure. The amino-acid sequence Arg-Gly-Asp (termed RGD), which can be recognized by the $\alpha_5\beta_3$ receptor and by at least ten additional integrins, has been used as a lead structure in the development of $\alpha_5\beta_3$ inhibitors. The split synthesis paradigm allows large combinatorial libraries to be produced rapidly, and has been used for the synthesis of a diacylhydrazine library and its biological evaluation (on a solid support) using a soluble $\alpha_5\beta_3$ integrin receptor[2].

A library of 990 compounds, synthesized from TentaGel Macrobeads™, was prepared in mixtures of 33. Of those compounds synthesized, active mixtures were identified upon incubation of the beads with soluble, biotinylated $\alpha_5\beta_3$ receptor and subsequently with a monoclonal antibiotin alkaline phosphatase conjugate. These mixtures were then deconvoluted and synthesized as single entities, this time from Rink amide (4-methylbenzhydralamine) resin. One of the most active compounds identified was **(iii),** which displayed an (IC_{50}) affinity for the $\alpha_5\beta_3$ receptor of 150 nM, with 48-fold selectivity over $\alpha_5\beta_5$ and >666-fold selectivity over $\alpha_{IIb}\beta_3$. This work illustrates the potential for discovering new, low-MW integrin ligands by the application of combinatorial solid-phase synthesis and biological on-bead evaluation.



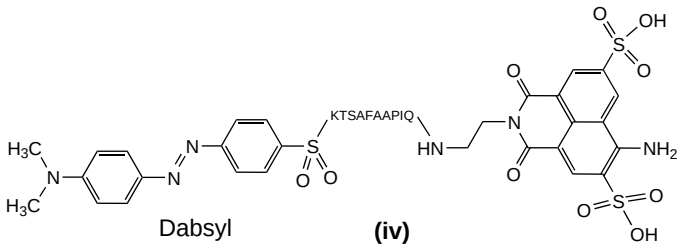
(iii)

2 Kessler, H. *et al.* (2001) Nonpeptidic $\alpha_5\beta_3$ integrin antagonist libraries: On-bead screening and mass spectrometric identification without tagging. *Angew. Chem., Int. Ed. Engl.* 40, 165–169

Substrates for novel proteases

A large amount of potential targets, of both human and microbial origin, for therapeutic intervention is expected to become available through genomics. One of the major challenges is to prioritize these targets. Homology with known genes and information about the location and temporal expression of the genes will be used in the prioritization process. Genes that are preferentially expressed in diseased tissue, compared with healthy tissues, are likely to be potential targets. Proteolytic enzymes represent one major class of targets because they are involved in a wide variety of disorders including infectious diseases and other pathologies. There is a need for optimal substrates to study enzymology of the selected proteases, for validation as relevant medicinal targets and to screen for inhibitors.

Sensitive fluorescence resonance energy transfer (FRET) experiments are extensively used in biomedical research for this purpose. A combinatorial chemistry approach can be used for the rapid identification of fluorogenic substrates, allowing for the development of HTS assays for the fast and reliable testing of compound libraries against an increasing amount of targets. The technology consists of a solid-phase enzyme assay system using polymer-bound peptide libraries built up by split synthesis, and is based on the principle of FRET[3]. A library of theoretical size 260,642 individual peptides, aimed at targeting leader peptidases (integral membrane proteins that catalyze the removal of the signal peptides of proteins exported to the periplasm) was synthesized on $PEGA_{1900}$



(iv)

solid-phase resin. Incubation of the beads with *Escherichia coli* leader peptidase gave bright fluorescent beads with active peptides. These were harvested and the active amino acids within these sequences identified to provide the exact site of enzyme cleavage on the peptide chain. Following deconvolution of active mixtures, and resynthesis of these peptides as single compounds, peptide **(iv)** was isolated and found to have the greatest affinity for the enzyme, with a K_m value of 0.43×10^{-6} M.

The benefits of this technology are that it can be performed on single beads and can rapidly access motifs within peptides that are recognized by enzyme substrates. It could also find use in determining fluorogenic substrates for novel, poorly characterized proteases.

3 Rosse, G. *et al.* (2000) Rapid identification of substrates for novel proteases using a combinatorial peptide library. *J. Comb. Chem.* 2, 461–466

Paul Edwards
Lead Discovery Technologies
Pfizer Global Research and Development
Sandwich
Kent, UK
fax: +44 (0)1304 643555
e-mail: paul_edwards@sandwich.pfizer.com

Contributions to *Monitor*

We welcome recommendations of papers for review within *Monitor*, in the fields of combinatorial chemistry, pharmacogenomics, pharmacoproteomics, bioinformatics, new therapeutic targets, high throughput screening, new drug delivery technologies and other promising lines of research. Details of recent papers or those *in press* should be directed to Dr Debbie Tranter, Editor, *Drug Discovery Today*, Elsevier Science London, 84 Theobald's Road, London, UK WC1X 8RR. tel: +44 (0)20 7611 4132, fax: +44 (0)20 7611 4485, e-mail: deborah.tranter@current-trends.com

Contributions to *Profiles*

We welcome contributions for the *Profiles* series, which gives a commentary on promising lines of research, new technologies and progress in therapeutic areas. Articles should provide an accurate summary of the essential facts together with an expert commentary to provide a perspective. Brief outlines of proposed articles should be directed to the *Monitor* Editor (see below). Articles for publication in *Monitor* are subject to peer review and occasionally may be rejected or, as is more often the case, authors may be asked to revise their contribution.The *Monitor* Editor also reserves the right to edit articles after acceptance.

All suggestions or queries relating to Monitor should be addressed to Dr Debbie Tranter, Editor, *Drug Discovery Today*, Elsevier Science London, 84 Theobald's Road, London, UK WC1X 8RR. tel: +44 (0)20 7611 4132, fax: +44 (0)20 7611 4485, e-mail: deborah.tranter@current-trends.com