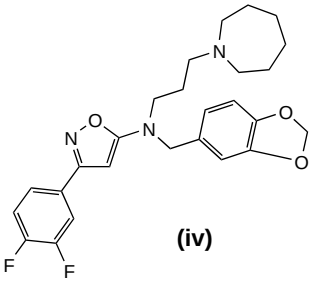
physiologically relevant thrombin. Some differences in SAR were observed depending upon the stimulus, with compound **iv** being identified as a potent antagonist in both assays (IC_{50} = 0.09 μM with TRAP as the agonist, 0.51 μM with thrombin and a binding IC_{50} of 0.15 μM measured by displacement of a radiolabeled peptide).



(iv)

Compound **iv** is not an inhibitor of the catalytic activity of thrombin, nor does it inhibit platelet aggregation induced by ADP. However, it does inhibit platelet aggregation induced by 1 nM thrombin for 10 min at a concentration of 4 μM and for 5–6 min at 1 μM.

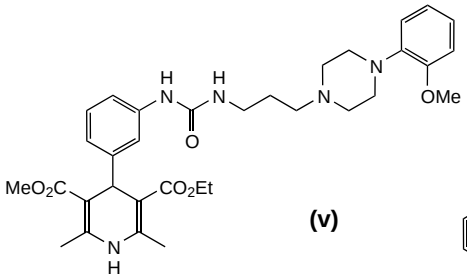
2 Nantermet, P.G. (2002) Discovery of a nonpeptidic small-molecule antagonist of the human platelet receptor (PAR-1). *Bioorg. Med. Chem. Lett.* 12, 319–323

Small-molecule neuropeptide Y receptor antagonist

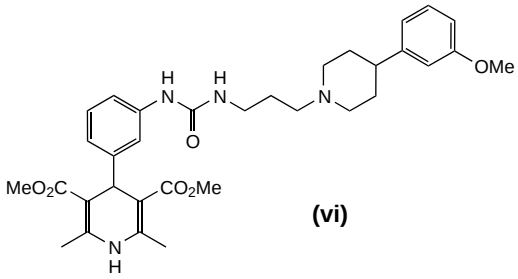
Obesity is a growing public health concern in the Western world. There are many factors that cause obesity, however, it is clear that its progression is closely associated with an imbalance between food intake and energy expenditure.

Neuropeptide Y (NPY) is a 36 amino acid peptide that is prevalent in the peripheral and central nervous systems, has been implicated in the regulation of feeding behavior and is the most potent stimulant of feeding known, to date.

There are six known NPY receptor subtypes with Y_1 and Y_5 being involved in the feeding response. HTS at the Bristol-Myers Squibb Pharmaceutical Research Institute (Wallingford, CT, USA) identified the dihydropyridine **v** as a



(v)



(vi)

competitive Y1 receptor antagonist with a K_i value of 109 nM [3]. The propyl side chain was found to be the optimum length and a piperidine ring could replace the piperazine. Replacing the ethyl ester with the smaller methyl ester improved binding and also had the advantage of rendering the dihydropyridine achiral. Compound **vi** was identified as competitive, full-functional antagonist with a K_i value of 3.3 nM at the Y_1 receptor and no affinity for the other NPY receptors. Intraperitoneal administration of **vi** at 10 and 30 mg kg^{-1} antagonized the increase in food consumption (33% ± 15% and 57% ± 11%, respectively) induced by ICV infusion of NPY in satiated rats. Compound **vi** was also shown to decrease spontaneous nocturnal feeding in the rat. The compound highlights the potential of Y_1 antagonists to treat obesity.

3 Poindexter, G.S. (2002) Dihydropyridine neuropeptide Y Y1 receptor antagonists. *Bioorg. Med. Chem. Lett.* 12, 379–382

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

Combinatorial chemistry

Nucleoside phosphoramidates

Compounds that have phosphoramidate functionality have a range of biological activities. Well known examples include the anticancer drug cyclophosphamide and the cardioprotective agent phosphocreatine. As phosphoric and carboxylic equivalents, phosphoramidates have been evaluated as analogues of nucleosides and oligonucleotides. For example, 5′-phosphoramidates have been synthesized as 'prodrug' derivatives of antiviral nucleosides, such as 3′-azidothymidine (AZT), reported to possess anti-HIV activity. Oligonucleotides with primary, secondary and tertiary phosphoramidate internucleotidic linkages have been evaluated as antisense agents. However, only a few nucleoside phosphoramidates have been prepared and evaluated for antiviral activity.

The clinically useful antiviral drugs target mainly viral reverse-transcriptase (RT), DNA polymerase and protease. However, two crucial issues have emerged from their therapeutic use: (1) the rapid development of drug resistance; and (2) side effects such as mitochondrial and bone-marrow toxicity associated with most polymerase and RT inhibitors. Newer strategies are, therefore, needed to combat viral infections.

Combinatorial synthesis and HTS has stimulated efforts to assemble novel libraries of compounds for evaluation against biological targets. This strategy, combinatorial synthesis combined with the screening of biologically relevant libraries for their ability to modulate biological pathways, with or without regard to specific molecular targets, is appropriate in the context of antiviral lead discovery. This approach enables the

 1359-6446/02/$ – see front matter PII: S1359-6446(02)02299-7

simultaneous identification of leads and the potential discovery of novel molecular targets. Phosphoramidates derived from NAB™ (nucleic acid-based) scaffolds can provide a variable spatial display of functionalities that facilitate hydrophobic, hydrogen bonding and ionic interactions with crucial viral protein and nucleic acid targets. These attributes make NAB™ phosphoramidates a novel source of biologically relevant diversity [1].

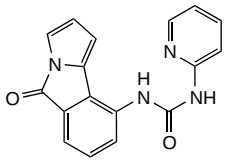
A library of 600 single compounds was synthesized in solution. The library compounds were screened at 10 μM against hepatitis B virus (HBV) in cell-based assays for antiviral activity. Several potent antiviral compounds were found and lead optimization is currently in progress to improve antiviral potency. This work has provided a library of nucleoside phosphoramidate compounds as a potential new class of anti-HBV agents.

1 Iyer, R.P. *et al.* (2001) Parallel solid-phase synthesis of nucleoside phosphoramidate libraries. *Bioorg. Med. Chem. Lett.* 11, 2057–2060

Cdk4 inhibitors

Cyclins and cyclin-dependent kinases (Cdks) play important roles in regulation of the cell cycle. In particular, D-type cyclins, which are activated by rearrangement or amplification in several tumours, associate with Cdk4/6. Cyclin D–Cdk4/6 complexes phosphorylate the retinoblastoma protein (pRB) and regulate the cell cycle during G1–S transition. Loss of function or deletion of $p16^{ink4a}$ (endogenous Cdk4/6 specific inhibitor protein) frequently occurs in cancer cells observed in the clinic. As the next generation of Cdk inhibitors, selective inhibitors of a single target Cdk are expected to specifically cause cell-cycle arrest. The suppression of tumour growth by G1 arrest is thought to reduce the stress for normal cells more than in other phases, because normal cells are usually resting in the G0–G1 phase. Thus, the design of Cdk4 selective inhibitors that cause cell-cycle arrest in the G1 phase has been attempted [2].

To obtain highly selective and potent Cdk4 inhibitors, a structure-based design was performed based on a Cdk4 homology model and enhancement of Cdk4 selectivity of lead compounds over Cdk1/2 and other kinases, based on the binding modes and structural differences between Cdk4 and other kinases. These criteria were used to search the Available Chemicals Directory and 382 commercial compounds were selected for screening in cyclin D–Cdk4 assays at concentrations up to 1 mM. From this set, 18 compounds were found that possessed an IC_{50} value of <500 μM. From these hits, a class of diarylureas were identified with the potential for follow up with parallel synthesis to validate the potential of the scaffold and to obtain preliminary structure–activity relationship (SAR) data. Based on the design of the diarylurea hits, 410 urea compounds were then synthesized as singles in solution and were screened in a Cdk4 inhibition assay. One of the most potent compounds isolated was **i**, which possessed an IC_{50} value of 34 nM. This work has used a structure-based lead generation approach consisting of homology modelling of the target protein, construction of a library of compounds, followed by modification of hits obtained based on the predicted binding mode. This strategy has provided potent compounds from a new class of diarylurea Cdk4 inhibitors and is a good foundation for further work to improve potency in this series.



(i)

2 Honma, T. *et al.* (2001) Structure-based generation of a new class of potent Cdk4 inhibitors: New *de novo* design strategy and library design. *J. Med. Chem.* 44, 4615–4627

Paul Edwards
Lead Discovery Technologies
Pfizer Global Research and Development
Sandwich, Kent, UK CT13 9NJ
fax: +44 1304 643555
e-mail: paul_edwards@sandwich.pfizer.com