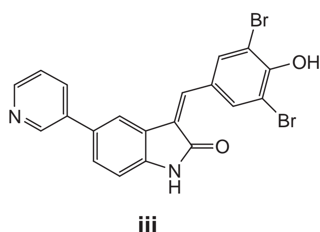
MEK (MAP kinase/ERK kinase), ERK2 (extracellular-regulated kinase) and the subsequent target identification of the potent cascade inhibitors.

As inhibition of cRAF1 kinase should cause down-regulation of MAP kinase activity, the group also used a cell-based mechanistic assay to measure the inhibition of endothelial growth factor-stimulated MAP kinase activation. This study identified several potent cRAF1 kinase inhibitors, exemplified by (**iii**), demonstrating low nanomolar kinase enzyme inhibition that also inhibited the MAP kinase pathway *in vitro*.



iii

2 Lackey, K. *et al.* (2000) The discovery of potent cRaf1 kinase inhibitors. *Bioorg. Med. Chem. Lett.* 10, 223–226

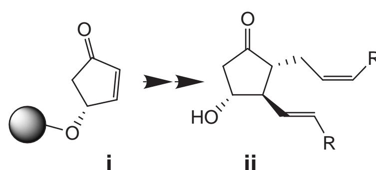
Andrew Lloyd

Combinatorial chemistry

Antiviral prostanoid libraries

The prostaglandin family constitutes one of the most pharmacologically active low-MW chemical classes in existence. A library of prostanoid analogues has been prepared on a soluble polymer support and used to find novel inhibitors of a herpes family virus[1].

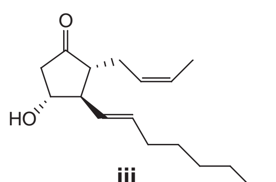
A 'parallel pool' library strategy was employed in which small pools of compounds were modified through several different functionalization reactions. Starting with the enone (**i**) attached to a soluble polystyrene support, several different ω- and α-chains were added to generate the library (**ii**). Cleavage of the products from the support using fluoride ions enabled biological evaluation of the library pools, and deconvolution



i **ii**

of active pools gave the identity of the active single analogues.

The library of prostanoids was screened for their ability to inhibit the replication of murine cytomegalovirus (CMV), and the analogue (**iii**) was discovered as the most potent compound.



iii

Although this compound is one order of magnitude less potent than ganciclovir (the most frequently used anti-CMV agent) this level of activity has encouraged the preparation of other structurally related prostanoids in a second generation library to further the search for more active agents.

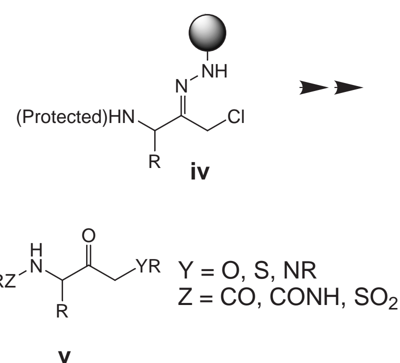
1 Lee, K.J. *et al.* (1999) Soluble-polymer supported synthesis of a prostanoid library: identification of antiviral activity. *Org. Lett.* 1, 1859–1862

Cysteine protease libraries

Cysteine proteases are characterized by the presence of a key nucleophilic cysteine residue in the active site of the enzyme that attacks the carbonyl of the hydrolyzed substrate amide bond. Various cysteine proteases are known to have physiological functions that make them suitable as targets for pharmacological intervention. Examples include calpains (implicated in neurodegeneration), cathepsin K (linked to osteoporosis) and caspases (which are possibly involved in apoptosis).

Various mechanism-based inhibitors have been designed, many of which depend on an electrophilic group such as a carbonyl or Michael acceptor that can react to form a covalent link to the nucleophilic thiol group of the crucial cysteine. A recent publication describes a versatile method of producing ketone-based cysteine protease inhibitors that permits maximal variation of the ketone structure[2].

A chloromethyl ketone, readily prepared from N-protected amino acids, was linked to the solid support by reaction with a hydrazine linker. The tethered carbazate (**iv**) was then derivatized by nucleophilic displacement of the chloride, and further derivitization of



the deprotected amine. Several products (**v**) were made, without racemization of the α-chiral centre, and they were ultimately liberated from the solid support with overall yields of 40–100%. Library preparation using this methodology is currently ongoing and the products will be evaluated against representative cysteine proteases.

2 Lee, A. *et al.* (1999) General solid-phase method for the preparation of mechanism-based cysteine protease inhibitors. *J. Am. Chem. Soc.* 121, 9907–9914

Orally active GPIIb/IIIa-receptor antagonists

Several serious cardiovascular conditions, such as unstable angina and myocardial infarction, are associated with

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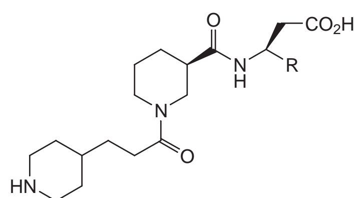
platelet aggregation, atherosclerotic plaque formation and, ultimately, thrombosis. In the process of thrombus formation, the final crucial step is the adherence of the protein fibrinogen to the activated membrane-bound glycoprotein GPIIb/IIIa. Compounds that can compete with fibrinogen for the platelet glycoprotein receptor are potential antithrombotic agents. In the search for orally active agents that have the potential to be used for chronic cardiovascular care, a group from the R.W. Johnson Pharmaceutical Research Institute have used combinatorial chemistry to optimize a prototype fibrinogen receptor antagonist[3].

Solid-phase parallel synthesis, employed in the preparation of approximately 250 analogues of compound (**vi**), led to the discovery of RWJ53308 (**vii**). This compound is an antiplatelet



agent that can be administered both intravenously and orally, and has a long duration of action. The compound has been successfully progressed through Phase II clinical trials.

3 Hoekstra, W.J. *et al.* (1999) Potent, orally active GPIIb/IIIa antagonists containing a nipecotic acid subunit. Structure–activity studies leading to the discovery of RWJ-53308. *J. Med. Chem.* 42, 5254–5265

Nick Terrett
Discovery Chemistry
Pfizer Central Research
Sandwich, Kent, UK
fax: +44 1304 655419
e-mail: nick_terrett@sandwich.pfizer.com

High-throughput screening

Evolution of Homogeneous Time Resolved Fluorescence (HTRF) technology for HTS

Increasing demand for drug candidates has led pharmaceutical companies to identify alternative strategies for improving the efficiency of HTS. One of the key technologies that has assisted the impressive development of HTS since its implementation is the use of fluorescence-based assays. In addition to being relatively inexpensive, the main advantages of these detection systems include sensitivity, versatility, stability, safety and ease of disposal.

However, two key problems are associated with the detection of fluorescence signals. Firstly, the presence of quenchers in the reaction can inhibit the signal generation. Secondly, autofluorescence from free probes or contaminant components can mask the signal. These limitations have been overcome to some extent by altering specific aspects of the fluorescent measurement such as the fluorescence lifetime, the energy transfer, or the anisotropy, enabling quench or autofluorescence correction adjustment.

The Homogeneous Time Resolved Fluorescence (HTRF) technology, developed by CIS-BIO International (Bagnols/Ceze Cedex, France, is one of the four main fluorescence-based technologies that are used in HTS (Ref. 1). The selectivity of the signal detection combined with the powerful data-analysis system enables not only the development of homogeneous assays but also the generation of a readout, corrected for the non-specific effects of the media.

Principle of the HTRF technology

HTRF uses a Eu^{3+} ion caged into a polycyclic cryptate (Eu–cryptate) as a donor. Laser exitation at 337 nm transfers the energy from the Eu–cryptate complex to an allophycocyanin acceptor molecule, the APC. This results in the emission of light at 665 nm over a prolonged time (milliseconds). This light emission is recorded in a time-resolved fashion over a 400 μs period, starting 50 μs after the excitation pulse so that the autofluorescence from the media and the short-lived fluorescence of the free APC are not recorded.

The modulation of the energy transfer depends on the distance between Eu–cryptate and the APC. The signal emitted by free molecules labelled with Eu–cryptate and APC is not recorded because they are not in close proximity during the required period of time. The Eu–cryptate emission peak at 620 nm is also recorded and used as an internal control. The data are expressed as a ratio of the fluorescent signal at 665 nm (coming from energy transfer between the two bound molecules) to the signal at 620 nm. This ratio of flourescence is proportional to the quantity of biological complexes present in solution (Fig. 1)[2].

The evolution of HTRF in HTS

Since its validation and implementation in HTS, the HTRF technology has undergone constant evolution (Fig. 2). Initial applications for the technique were developed using classical biochemical formats to assess, for example, binding of epidermal growth factor (EGF) to its receptor, the JUN/JOS protein–protein interaction, and for use in various enzymatic assays[2].

The development of generic formats for HTRF biochemical assays became a necessity as the demand for higher throughput in HTS increased and there was more dependence placed on the vendor for the labelling of reagents and for expertise. A complete HTRF generic test requires:

- Generic tools such as anti-tag antibody or streptavidine linked to the cryptate or APC tracers that will recognize the tagged proteins or the biotinylated antibodies
- Generic protocols
- Generic formats for a whole family of

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