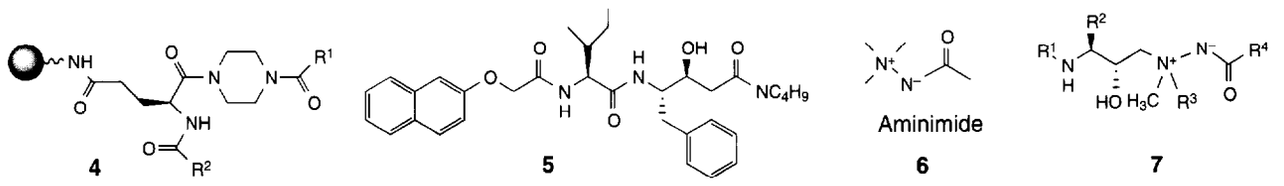

Figure 4. *Synthelabo's glutamine piperazinyl scaffold anchored to a solid-phase support via the glutamine side chain (**4**); Pharmacopeia's plasmepsin I inhibitor (**5**); general structure of aminimides (**6**) and ArQule's aminimide library (**7**).*

(Figure 4). Functionalization of the amines gave a library of 1,296 compounds after cleavage, from which nonpeptide IL-1 antagonists with sub-micromolar activity were identified.

A related combinatorial approach was described by Dr Hitesh Patel (Pharmacopeia, Princeton, NJ, USA) for identifying selective inhibitors of the aspartic proteases cathepsin D and the plasmepsins. Aspartic protease inhibitors usually contain a transition state mimetic core consisting of a secondary hydroxyl group β to a residue that binds in the S1-pocket of the protease. The hydroxyl group displaces the water molecule required for amide hydrolysis from the enzyme's active site. The invariant hydroxyl was linked to the resin using a photolabile linker and the amine and carboxyl termini functionalized by reacting with acids, aldehydes or amines to give a 20,000-compound library. Deconvolution was aided by the use of tagging methodology, and useful leads were identified especially for plasmepsin I. Compound **5** (Figure 4) exhibited low nanomolar inhibition of plasmepsin I with 100-fold selectivity over plasmepsin II and 10,000-fold selectivity over cathepsin D.

An unusual class of compounds that have found success as peptidomimetics are the aminimides of general structure **6** (Figure 4). Dr Steven Gallion (ArQule, Medford, MA, USA) described the synthesis of aminimide libraries (**7**) for the inhibition of HIV protease and elastase. The hydroxyaminimides for HIV protease inhibition were synthesized by a one-pot acylation of a substituted hydrazine and ring opening of an epoxide. Aminimides have potential as future drugs; they exhibit oral bioavailability, low toxicity and are present in known pharmaceuticals, such as besulpamide.

The above report can only hope to give a flavour of the conference. My apologies to those speakers whose presentations have not been reviewed.

Steven Langston
Peptide Therapeutics
321 Cambridge Science Park
Milton Road
Cambridge, UK CB4 4WG
fax: +44 1223 423111
e-mail: stevelangston@peptide.co.uk

Natural products drug discovery – new technologies and approaches

A two-day meeting entitled *Natural Products Drug Discovery: new technologies to increase speed and efficiency* was held in San Diego (CA, USA) on 17 and 18 March 1997. The purpose of this meeting, organized by IBC, was to discuss how natural products research within the pharmaceutical industry could continue to become more efficient and innovative in order to maintain its position as a valuable source of drug leads. One of the three sessions, 'New Technologies to Expedite Discovery', addressed this core issue, with the remaining two discussing 'New Drugs from Natural Products', and 'Biodiversity and Supply'. There was a very useful post-conference workshop, which addressed the 'interfacing' of combinatorial and natural product technologies. This report summarizes some of the key points of interest that were discussed at the conference and the workshop.

New technologies to expedite discovery

The rate-limiting step in natural products screening programs has traditionally been the isolation and characterization of bioactive metabolites from relatively crude and uncharacterized natural extracts. Intensive efforts have been made to increase efficiency here; indeed some companies have opted to 'prefractionate' such complex source extracts to provide purer samples that can be more rapidly characterized following the detection of activity in a screen.

Dr Ines Chicarelli-Robinson (Xenova, Slough, UK) presented Xenova's initiative to generate pure compounds for screening. The process comprises an initial HPLC/UV/MS analysis of a crude plant or microbial fermentation-broth extract. After searching the appropriate

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spectral databases – to identify and exclude known compounds – chromatographic peaks are selected for scale-up isolation and inclusion in a sample library for screening. The objective is to provide multi-milligram quantities of more than 10,000 pure compounds (>90% w/w) over a five-year period. Certain physicochemical characteristics are recorded for all samples (for example polarity, aromaticity index, functional group data and molecular weight), and full structures are elucidated for a proportion of the samples for quality control purposes. If activity is detected in a sample, its chemical structure is elucidated within one month.

Sample profiling

Sample profiling techniques were discussed by Dr Yue-Zhong Shu (Bristol-Myers Squibb, Wallingford, CT, USA). At Bristol-Myers Squibb (BMS), all bioactive natural product samples undergo detailed profiling before isolation work starts. The objective is to obtain information on compound novelty, to filter out false positives and interfering materials, and to allow standardization of protocols and hence automation where appropriate. For profiling of fermentation-derived samples, initially several solvent extracts are taken (e.g. butan-1-ol, ethyl acetate, butanone). Extracts are then subjected to cation/anion exchange and HP-20 (styrene-divinylbenzene copolymer) fractionation. All extracts and derived fractions are assayed, and the most active fractions are further fractionated by HPLC with UV/MS detection (using one of three generic systems for polar, moderately polar and nonpolar fractions). Following a bioassay, active peaks are searched against several databases [*Derep 1* (BMS in-house second derivative UV library with 600 entries), *BERDY, Chapman and Hall, Antibase, STN registry file (CAS)*]. For the extraction of plants different solvents are used (primary extraction with aqueous methanol; extract partitioned with e.g. hexane, chloroform/methanol). In addition, polyamide cartridges are used as a tannin screen, and a further extract of source material is obtained using supercritical fluid extraction. This approach has reduced the overall time taken to characterize the active component within a complex sample from six months to about three.

Sepbox

Dr Lutz Muller-Kuhrt (Analyticon AG, Berlin, Germany) gave an impressive account of a fully automated natural product isolation system – the Sepbox. A crude extract (1–5 g) is adsorbed onto a precolumn, then fractionated by preparative reverse-phase gradient HPLC. The effluent is diluted with water, and time slices are 'trapped' by adsorption onto cartridges containing a polymeric adsorbent. After elution, fractions are further chromatographed using 1 of 6 HPLC methods and time slices are collected and trapped in the same way. Finally, fractions (corresponding to discrete peaks by analytical HPLC with light scattering detection) are eluted into vials for screening. All this is achieved in less than 24 hours, and the output is up to 300 'almost pure' compounds in 0.1–100 mg quantities. Solvent consumption is substantial (200 l), but nearly all is recycled.

New drugs from natural products

Dr Mahesh Patel (Schering-Plough Research Institute, Kenilworth, NJ, USA) described the discovery and early phase development of novel everinomicin antibiotic *Ziracin* (Sch27899; **1**; Figure 1) from *Micromonospora carbonacea*. Ziracin, an oligosaccharide-like structure containing eight sugar residues esterified at either end with highly substituted benzoic acids, was discovered in 1979 and shown to have both potent Gram-positive antibacterial activity and low nephrotoxicity, but it was not progressed because of the small market potential of a narrow spectrum (Gram-positive) antibacterial. With the emergence of multiple drug-resistant Gram-positive

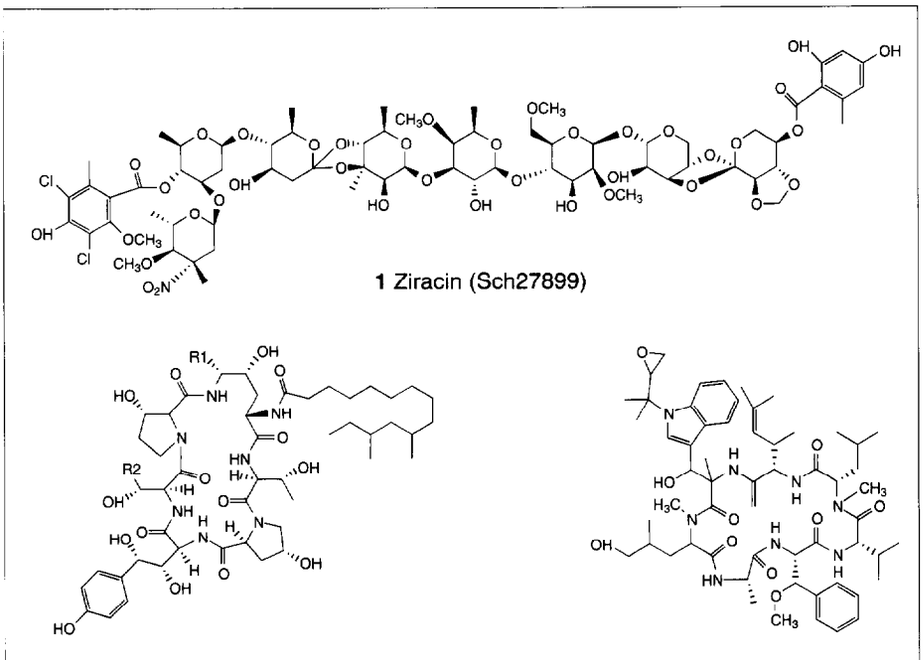

Figure 1. *Schering-Plough's novel antibiotic Ziracin (**1**); Merck's broad-spectrum antifungal agent L743872 (**2**), derived from fungal metabolite pneumocandin B_0 (**3**); anti-inflammatory natural product cyclomarin A (**4**) isolated from a sediment actinobacterium.*

pathogens, times have changed and the compound has now entered phase I clinical trials. It is more active than vancomycin against methicillin-resistant *Staphylococcus aureus* and highly active against both vancomycin-resistant enterococci and penicillin-resistant *Streptococcus pneumoniae*, and does not show cross-resistance with any other class of antibiotics. Ziracin is produced by a two-stage germination and two-stage fermentation process, and isolated by ethyl acetate extraction of whole broth followed by multiple cycles of silica gel chromatography and precipitation.

Antifungal agents

Dr Steve Gould (Merck) argued that 'all microorganisms offer all possibilities', and that the limiting factor in the discovery process is the suitability of high-throughput assays for crude natural product extracts rather than the nature of the extracts themselves. An overview of the substantial natural product discovery operation at Merck was presented, followed by a discussion of a promising antifungal agent L743872 (**2**; Figure 1), which is in phase II clinical development. The initial drug lead was the fungal metabolite pneumocandin B_0 (**3**; Figure 1). Unlike other pneumocandins, the compound lacked toxicity, but was highly insoluble, possessed only moderate antifungal activity and was not active against *Aspergillus*. A medicinal chemistry program followed, resulting in the discovery of the clinical candidate L743872. This differs structurally from the natural product at two positions of substitution around the pneumocandin ring system: an amide is reduced to a primary amine, and an aliphatic hydroxyl is replaced with a 1,3-diaminoethyl group. The semi-synthetic derivative has greater water solubility (>200 mg/l), improved pharmacokinetics, and both increased potency and an extended spectrum of activity which includes *Aspergillus, Candida* and *Pneumocystis carinii*.

Biodiversity and supply

Dr William Fenical (Scripps Institute, La Jolla, CA, USA) gave an interesting account of the chemical diversity found in marine microorganisms. Though sea water typically contains a few million microorganisms per milliliter, most are poorly understood and it is estimated that less than 2% of observed microorganisms have been cultured successfully in the laboratory. Despite this, the chemistry unearthed so far looks impressive. A range of structural types including cyclic peptides and depsipeptides, macrolides, and various oxygen and nitrogen-containing heterocycles, including a significant proportion of halogenated metabolites, were presented. The cyclic peptide *cyclomarin A* (**4**; Figure 1), isolated from a sediment actinobacterium (CNB382) was found to have good *in vitro* and *in vivo* anti-inflammatory activity (acting via inhibition of arachidonic acid synthesis), and is in clinical development.

Dr Christine Moller (Sandoz, Basel, Switzerland) focussed on ecological approaches to the assessment of chemical diversity. Since the 1940s millions of microorganisms have been screened for lead discovery from a vast array of habitats, but little reliable information is available on how environmental factors account for metabolic 'creativity' of these microorganisms. The aim of the Sandoz investigation was to reveal possible correlations between environmental factors and metabolic creativity, so that microorganism isolation and screening programmes could pick the richer 'fishing grounds'. A large number of microorganisms were isolated from a variety of environments from polar through to temperate, alpine and tropical. Organisms were morphologically and taxonomically classified, and then cultured. Methanol extracts were subjected to bioassays versus selected fungi, Gram-positive and Gram-negative bacteria, and to analytical scale gradient HPLC. The data were then combined to give a three-dimensional 'eco-taxonomic-metabolic' landscape. In the example given, soil penicilli appeared to be very creative (the mountains on the landscape), whereas yeasts were very unproductive (the plains).

Interfacing combinatorial and natural products technologies

Dr Reimar Bruening (Millennium Pharmaceuticals, Cambridge, MA, USA) considered natural product extracts to be essentially the same as pooled combinatorial library samples, and outlined Millennium's approach to the characterization of activity in such samples using a combination of LC, MS and NMR techniques. Of considerable interest was the approach to rapid generation of structure–activity data around a compound of interest, using affinity capture coupled to LC-NMR. The starting point for this procedure is an extract with an at least partially characterized active component. Initially, this extract is analysed by HPLC-NMR in order to localize the component of interest together with other related components. It is then incubated with the target protein (e.g. a soluble receptor). After a size-exclusion step, material that is not bound to protein is subjected to an identical HPLC analysis, and the peak integrals from the two chromatograms are compared. In this way, the strength of interaction of active components in an extract to a target protein can be estimated by differences in peak areas before and after incubation. This, coupled to LC-NMR data, can provide structure–activity information 'on the fly' without full isolation, structure elucidation and biological evaluation of individual components using traditional methods.

Polyketides and combinatorial biology

Polyketides, particularly macrocyclic lactone derivatives such as erythromycin, have long been of considerable medicinal importance. Recent advances in the understanding of their unique biosynthesis has caused renewed excitement in this field, because of the possibilities for generating large libraries of novel macrocycles through rational manipulation of the biosynthetic genes. Two presentations reviewed the current state of knowledge of actinomycete (and to a lesser extent fungal) polyketide biosynthesis. Dr James McAlpine (Phytera, Worcester, MA, USA) gave a thorough review of the actinomycete

polyketide synthase (PKS) pathway, focussing on the 14-membered macrolide erythromycin.

Dr Mary Betlock (Kosan Biosciences, Burlingame, CA, USA) reviewed the biosynthesis of macrocyclic polyketides, but also talked of the combinatorial potential of the aromatic PKS pathway, citing some of Khosla and Canes work on the mixing and matching of PKS genes to generate novel isochromanequinones.

Dr David Sherman (ChromaXome/Houghten Pharmaceuticals, San Diego, CA, USA) gave a stimulating talk on ChromaXome's approach to generating and screening natural pathway and combinatorial expression libraries of natural products. The natural pathway library process involves isolating genomic DNA from a pool of cultivable donor species or from an environmental sample, ligating large fragments into suitable vectors, then introducing these into a well characterized expression host possessing a constant, low background. The combinatorial approach firstly identifies clones (from a cosmid library made from pooled donor species) using hybridization with known biosynthetic genes. Cosmid DNA is isolated, partially digested, and size-selected fragments randomly ligated together. DNA is then redigested to generate fragments of 30–40 kb in length, ligated into appropriate vectors, and introduced into appropriate expression hosts as before. In this manner, it is anticipated that novel compounds will be generated through the hybrid combinatorial pathways that are created and expressed. This approach to lead discovery was tested in a collaborative project with BMS. Only limited details were presented, but it was noted that from 10,400 streptomycete clones (8,200 natural pathway, 2,200 combinatorial pathway) tested for biological activity (antimicrobial – Gram-positive and -negative bacteria, yeast, filamentous fungus – and DNA reactivity), potential novelty was seen in all hits examined chemically, comparing them with the BMS natural products database.

A novel approach to encapsulation and screening of transformants was presented: cells are dispersed in a medium containing sodium alginate, which is then added dropwise to a solution of calcium alginate. Sodium ions are traded for calcium ions causing the alginate to harden. By encapsulating a transformant together with a cellular reporter gene-based assay system, single bead screening of such transformants becomes a possibility.

Finally, Dr Alan Chumurny (Oceanix Biosciences Corporation, Hanover, MD, USA) outlined the Oceanix strategy for accessing secondary metabolites from 'nonculturable' microorganisms as sources of novel pharmacophores and lead compounds. The objectives of the program are firstly to define DNA extraction/preparation procedures that effectively access the microbial genetic diversity in environmental samples, and secondly to establish genetic systems for the transfer and expression of DNA coding for large biosynthetic pathways into culturable host microorganisms. To date, direct DNA extraction from marine sediments has been established with an average fragment size of >150 kb. Fragments have been packaged successfully into liposomes and lipofected into the host *Streptomyces lividans*. Transfer of multigene pathways has also been demonstrated. Experimental data from work undertaken with an actinomycin D-producing donor strain demonstrated that many *S. lividans* transformants could be generated possessing antibiotic activity, though different to actinomycin D. HPLC analysis of fermentation broth extracts of control and transformed strains revealed significant differences, as well as absence of actinomycin D production. New chromatographic peaks might correlate with actinomycin D fragments or perhaps cryptic pathway expression products.

Paul Stead
Natural Products Chemistry Group
Bioprocessing Unit
Glaxo Wellcome
Medicines Research Centre
Gunnels Wood Road
Stevenage, UK SG1 2NY
fax: +1 44 1438 764 473
e-mail: ps29705@ggr.co.uk