

Looking for natural products that are active against human pathogens

The growing concern about resistant bacteria and the need for new effective drugs to treat parasitic diseases in the developing world was the focus of a recent meeting. **Joseph Chamberlain** reports some of the highlights

The search for drugs from natural products is often seen as being in competition with drug discovery using the combinatorial chemistry approach, said Liam Evans, of Hypha Discovery Ltd, in an introductory lecture. Combinatorial chemistry seduces managers with the promise of more drugs discovered more quickly, but has a poor success rate and provides little structural diversity. The obsession with large numbers often obscures the benefit of careful observation. The recent history of natural product research fares little better. Routine processing of source material may mean suboptimal handling leading to compromised stability. Dereplication — the process of eliminating already known entities — is necessitated by lack of intelligent selection of source material. Novelty is low with bioactive molecules produced using non-specific fermentation techniques and focused on products from known plants and microbes.

There has been a slight improvement in recent years with more discovery work out-

sourced to biotech companies, but there is still the obsession for high numbers of extracts or organisms assessed. The desire in the pharmaceutical industry to return to natural product drug discovery must be accompanied by a drastic improvement in productivity. This is more likely to come from academia, with its high concentration of skilled natural products researchers, and the emphasis on quality through thorough chemical investigation and good biological characterisation. High throughput is not an issue, but academics are judged by their publications, not by their discovery of lead compounds.

The Convention on Biological Diversity also deters the pharmaceutical and biotech industries, with unrealistic commercial demands from developing countries, inflexible negotiators in the industry, and entrenched positions from the outset. Academics are less likely to be deterred, being one step further from the revenue implications, concentrating instead on technology transfer and exchange

programmes. The ultimate benefits from natural product research in academia include the higher chance of discovery of compounds for licence with revenue for both the university and the developing nation, conservation of threatened biodiversity, training for drug discovery PhD students, and worthwhile publications, concluded Dr Evans.

Is there really a case for using complex plant extracts as antimicrobial agents, asked Jacobus Eloff, of the University of Pretoria. He believed that looking to ethnopharmacology may be misleading, particularly if we wish to find novel useful structures. Possibly promising antibacterials have not been found because many scientists only used ethnobotanical leads and ignored plants that are not used medically, even though compounds with high activity have been found in non-medicinally used species. There is still considerable potential in new medicinal agents from plants, but they must be investigated intelligently and scientifically, concluded Professor Eloff.

Finding natural killer systems

Grant Burgess, of the University of Newcastle on Tyne, stated that there was a real possibility of finding viable pharmaceutical products in marine organisms, the ocean being the most chemically diverse environment on the planet.

Marine organisms have been devising ways of killing each other for billions of years. This experience should be taken advantage of, seeking to find killer systems which can be adopted as useful antibacterials. An understanding of the mechanisms involved will also help identify new targets for successful intervention or to develop variations on lead compounds discovered. As examples, Dr Burgess cited the disruption of cell-cell signalling systems that allowed organisms to communicate and coordinate action — a classic military strategy. If bacteria are attacked one line of defence is to create a protective slime. To break this biofilm other organisms will have developed appropriate chemicals and this is a likely source of effective antibacterials, said Professor Burgess.

Franz Hadacek, of the University of Vienna, stated that at present there were few antifungal compounds from plant sources. He suggested this may be because in general screening procedures, biological activity is rare, chemical diversity includes many inactive compounds as the plant apparently maintains options for future chemical strategies, and enzymes in the degradative pathways have little substrate specificity. Nevertheless there is considerable interaction between plants and fungi and study of the interactions should reveal agents in the plants that defend them from attack by fungi and hence may form the basis of antifungal products.

Natural sugars as medicines

Andreas Hensel, of the University of Muenster, explained that many pathogens need adhesion to host cells or tissues as a prerequisite for invasion and virulence. Adhesion is highly specific via a receptor-mediated adhesin-receptor interaction, and a new target may be hypothesised to block carbohydrate-mediated adhesion by exogenous carbohydrates. Using suitable test systems such as *Helicobacter pylori* against stomach tissue sections, a series of about 150 oligosaccharides, polysaccharides, heparins, mucins, peptides, glycoproteins allowed classification into those having no effect on adhesion, those causing increased adhesion and those showing decreased adhesion, including sialyl-lactose, fucoidan, and polysaccharides of raw okra.

Plant-derived polysaccharides, particularly strongly acidic ones, exhibit strong anti-adhesive effects. They are usually well-tolerated, but there may be an issue of *in vivo* activity after gastrointestinal application. Nevertheless, concluded Professor Hensel, antiadhesives derived from plants may be a potent tool for future bacterial prophylaxis.

Imino sugars are analogues of monosaccharides or disaccharides with the ring oxygen replaced by nitrogen, explained Robert Nash, of MNLpharma. Their modes of action include glycosidase inhibition and immunostimulation. Applications of imino sugars include priming of the immune response with wide ranging protective and healing effects, direct antiviral activity, and indirect antiviral, anticancer and antibacterial activity. The first generation of imino sugars (castanospermine and 1-deoxynojirimycin) caused GI disturbance at anti-viral doses. However, many others are consumed daily without obvious side effects and some may be the elusive active components of many herbal products. MNLpharma is finding many with improved specificity and modifications have shown therapeutic activities are possible without glycosidase inhibition. Full structural elucidation can be difficult but is the key to understanding activity, said Professor Nash.

This two-day meeting of the **Academy of Pharmaceutical Sciences** took place at the Jodrell Laboratory, Kew Gardens, London, on 6 and 7 April

Natural products to overcome drug resistance

The problem of methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug resistance is increasing despite public health campaigns, said Simon Gibbons, of the London School of Pharmacy. We should look to the mechanisms of developed resistance to solve this problem. Many clinically resistant bacteria have evolved efflux mechanisms. Reducing the concentration of antibacterial in the cell reduces the effectiveness so that one way of reducing resistance is to develop efflux pump inhibitors (EPIs) to be used in combination with an antibiotic in much the same way as clavulanic acid is used to reduce beta-lactamase activity of antibiotics.

Efflux of a fluorescent substrate has been used as a screening tool to investigate plants, plant extracts and existing libraries. Green tea metabolites are potentiators of methicillin against MRSA and this may be due to the weak EPI property of epicatechin gallate and epigallocatechin gallate. Ergotamine is a promising lead. It potentiates norfloxacin against an effluxing strain, it is not itself antibacterial and analogues are readily synthesised.

When evaluating pine metabolites as antibacterials it was discovered that the antibacterial activity of abietic acid was reduced in the presence of reserpine (a multidrug-resistant EPI), the reverse of what was expected. This suggests an interaction between a proven EPI and the antibacterial, a proposal supported by NMR and molecular modelling studies. There are great opportunities for new antibacterials and EPIs from plants, provided that the specificity and mode of action is understood, concluded Dr Gibbons.

Tuberculosis is the world's leading cause of death from a single pathogen, said Dr Veronique Seidel, of Strathclyde University. TB may be latent or active. In healthy subjects, im-

mune cells surround infected macrophages. In immunocompromised subjects bacilli overwhelm the immune system and spread to other organs. New drugs are required to reduce the duration and complexity of current therapy (and hence increase compliance), improve treatment of multidrug-resistant TB, and control latent TB. However few truly novel antimycobacterial agents have been introduced into clinical practice in the past 30 years, although there is a formidable biological diversity and a reservoir of natural products of great chemical diversity, with anti-TB metabolites having been isolated from microbes and marine organisms and from plants. An effective South African herbal remedy is the root known as umckaloabo. Bioassay-guided fractionation of this and other medicinal roots suggest that long chain unsaturated fatty acids may be responsible for antimycobacterial activity with the activity depending on the degree of unsaturation or ionisation. However, the precise mode of action is unclear, said Dr Seidel.

Apart from the continuing need for new and better drugs, increasing resistance of HIV towards existing synthetic drugs, makes the search for drugs from natural sources important, said Paul Cos, of the University of Antwerp. Additionally, synthetic drugs from the developed world are often too expensive for developing countries and the local natural products may have an economic attraction. Thus there is a search for new leads with new mechanisms of action, exploiting the chemical biodiversity of the plant kingdom. A complementary anti-HIV strategy was proposed with a viral target and a cellular target. Viral targets of current drugs are HIV fusion inhibitors (enfuvirtide), reverse transcriptase inhibitors (zidovudine, nevirapine) or protease inhibitors

(ritonavir). Viral targets for plant-derived agents can additionally include HIV attachment inhibitors (sulphated polysaccharides), cell fusion inhibitors (lectins and triterpenes), and some terpenes representing a new class of anti-HIV drug candidate, maturation inhibitors. Cellular targets include inhibition of cellular transcription factors, or cytokine production, antioxidants (flavonoids, proanthocyanidins), and inhibition of enzymes such as alpha-glucosidase by nojirimycins.

Recent developments in naturally derived antimalarials, particularly those related to artemisinin and cryptolepine were described by Colin Wright, of the University of Bradford. In the past 20 years mortality from malaria has doubled. A major factor is the development of malaria parasites resistant to chloroquine. The essence of modern antimalarial treatment is to include a second drug, often derived from artemisinin in normal therapy to stop recrudescence and delay resistance development. Combinations available or being developed include artemether-lumefantrine, artesunate-amodiaquine, and artesunate-mefloquine. The root extracts of *Cryptolepis sanguinolenta*, known as nibima, are traditionally used in Ghana for the treatment of fever, malaria, upper respiratory tract and urinary tract infections, sexually transmitted diseases, and gastrointestinal disorders. The active alkaloid is cryptolepine, which has potent activity against both chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum*. It is easily extracted from the roots of *C. sanguinolenta* and the synthesis of a wide range of derivatives is feasible. Derivatives and analogues with antimalarial activity have been prepared, some of which may have different modes of action against the parasite, said Dr Wright.

Possible uses for photodynamic antibacterial chemotherapy

The basis of photodynamic antibacterial chemotherapy (PACT), said Dave Phoenix, of the University of Central Lancashire, was that certain structures could absorb light and remain in an excited state long enough to provide the energy to generate free radicals which may cause photooxidative cell damage. Such structures include hypericin from St John's wort, hypocrellins from plant moulds, and aminolevulinic acid (ALA). Oral administration of ALA to patients positive for *Helicobacter pylori* plus laser light to the gastric antrum killed high numbers of the organism and there is great potential for clinical development. ALA-mediated PACT is also effective in removing blockages from the sebaceous duct — thus providing a two-fold action against acne.

Phenothiazinium-based compounds such as methylene blue show the potential to photoinactivate azole-resistant strains of *Candida albicans* in AIDS-related oral candidiasis in humans, and can inactivate HIV.

Methylene blue has been patented as a photodynamic disinfectant of blood and is widely used by a number of European transfusion services in the photodecontamination of blood plasma.

Active compounds in garlic

Garlic-based remedies have been used for at least 5,000 years for a variety of ailments. Ron Cutler, of the University of East London, described the latest research on allicin, one of its bioactive ingredients, against methicillin-resistant *Staphylococcus aureus* (MRSA). Pure allicin — which arises by conversion of alliin in garlic — is highly volatile and poorly miscible in water and a patented cold aqueous extraction method is used to prepare a stable aqueous allicin solution. Allicin has strong sulphhydryl-modifying and antioxidant properties, and reacts rapidly with free thiol groups, via a thiol-disulphide exchange reaction. The main antimicrobial effect of allicin is probably due to this reaction with the thiol groups of alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase. In *in vitro* studies, over 200 clinical isolates from hospital patients and from patients with chronic MRSA infections were tested and zones of inhibition for allicin liquids against MRSA strains were shown. In volunteer studies, patients on garlic extract treatment have generally reported an improvement in their condition after two and six weeks, with infections resolving in three to four months. The German Commission E — a therapeutic guide to herbal medicines — reports no side effects of garlic although some sources suggest that substantial amounts of garlic should not be consumed before surgery, since it can prolong bleeding time.