Australian microbial biodiscovery: from bugs to drugs

Robert J Capon

Division of Chemistry and Structural Biology, Institute for Molecular Bioscience
The University of Queensland, Carmody Road, St Lucia QLD 4072
Tel (07) 3346 2979
Fax (07) 3346 2090
Email r.capon@uq.edu.au

To maintain and improve the quality of life offered by modern healthcare requires an ongoing commitment to the development of new drugs, to improve and replace those that have become less effective, and to bring to the community safer treatments for an ever-wider array of important diseases. Irrespective of the specific medical need, the drug discovery pipeline is critically dependent on access to diverse, high-quality molecular libraries capable of inspiring drug-led discovery, and ultimately new drugs. A poor choice of chemistry leads to wasted resources and no drugs. Historically the pharmaceutical industry has relied heavily on microbial natural products, which represent an extraordinarily diverse, preassembled pool of biologically active molecules, programmed to be potent and selective modulators of key biopolymers, cells, tissues, organs and animals. Knowledge of Nature's intellectual property, gleaned from the evolutionary equivalent of a billion-year global drug discovery program, with an unlimited budget and a workforce of trillions, can disclose privileged bioactive structures that inform, guide and inspire modern drug discovery, re-purposing ecological advantage to pharmaceutical benefit.

After more than 70 years as the mainstay of the pharmaceutical industry, microbes have clearly demonstrated their capacity to produce multiple classes of valuable bioactive metabolites. For example, actinomycetes have supplied >50% of all antibiotics in use today, including erythromycin, vancomycin, aminoglycosides, tetracyclines and amphotericins, as well as important anticancer and immunosuppressive agents, and anthelmintics. Fungi have also been significant producers of important drugs, with the genus *Penicillium* yielding arguably the most well recognised name in modern drugs, the antibiotic penicillins, along with the most commercially successful drug class in history, the antilipidemic statins. The historic impact of microbial metabolites on human health has been profound. The journey to discover, isolate, identify, evaluate, develop, manufacture and deliver these metabolites to the market has sparked and fuelled a revolution in global science, commercialisation and healthcare. These successes notwithstanding, by the late 1980s economic considerations caused the pharmaceutical industry to question the future earning potential of microbial drug discovery, prompting a shift in strategy to such technologies as combinatorial chemistry. This reliance on synthetics was not without challenge and ultimately coincided with a period of record high R&D expenditure, record low numbers of new chemical entities (NCEs equate to new drugs), and a seriously depleted drug discovery pipeline (Figure 1). These outcomes (or lack thereof) present a compelling case to revisit and reappraise microbe-inspired drug discovery. Fortunately, modern technologies and methodologies have overcome the perceived shortcomings of the 1980s and are more than capable of revitalising microbial biodiscovery as a premier drug discovery paradigm.

Key advances have been made across multiple disciplines. Advances in genomics have, for example, documented and expanded the intrinsic molecular potential of the microbial genome, exposing a capability far exceeding that revealed during traditional fermentation. As the pace and extent of sequencing grows, a wealth of 'silent' secondary metabolism gene clusters emerges, coding for an untapped reserve of chemical diversity. Advances in microbiology have led to the discovery of metabolite rich marine-obligate actinomycetes, vastly expanding the pool (oceans) of microbial diversity available for drug discovery. Advances across biology have revealed knowledge of signalling, transport and developmental pathways that have led to a bewildering array of novel molecular targets, keyed to specific diseases. Advances in structural biology have visualised the intimate inner workings of the substrates and receptors that make up these targets, highlighting functional homology beyond peptide sequences and across species. High-sensitivity, high-

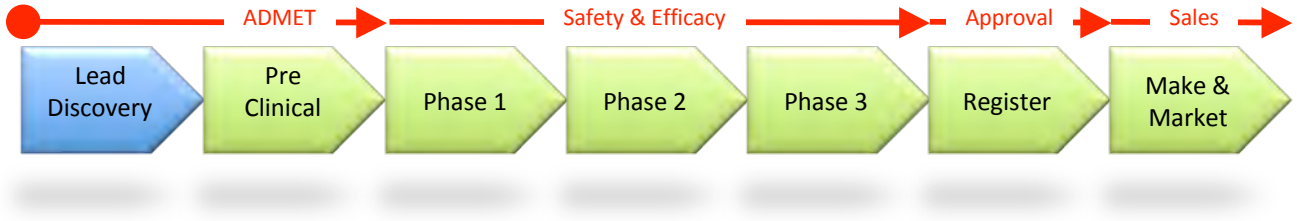

Figure 1. Drug discovery pipeline.

throughput and high-content bioassay technologies are driving the rapid discovery of potent and selective bioactive molecules. Advances in chemistry have returned unparalleled sensitivity and scope for detecting, isolating and identifying not only the major metabolites (as was the norm only two decades ago), but the full array of structurally related minor co-metabolites – Nature's equivalent of combinatorial chemistry. Empowered by ready access to such pre-programmed bioactive chemical diversity, structure activity relationship (SAR) investigations by co-metabolite have become a powerful paradigm for accelerating the evaluation and characterisation of new drug classes (pharmacophores). When applied to microbial biodiscovery, all these advances (and many more) lay bare a remarkable, untapped molecular potential. The following selected examples (Figure 2) are illustrative of this potential.

Calicheamicins (**1**) – First reported in 1989 from the bacteria *Micromonospora echinospora* [1], the anticancer 'enediynes' bind with DNA and initiate strand scission through an exquisite radical mechanism. With only a dozen or so natural enediynes identified to date, and commercial syntheses uneconomic, further development of this pharmacophore would be greatly assisted by access to new natural analogues. Encouragingly, a 2003 study [2] describing a genome scanning for the enediyne polyketide synthase (PKS) across 50 actinomycetes identified the PKS cassette in eight samples, reinforcing the potential to further explore this pharmacophore through microbial biodiscovery.

Daptomycin (**2**) – Isolated from *Streptomyces roseosporus,* this lipopeptide antibiotic disrupts multiple aspects of bacterial cell membrane function and is effective against serious infections caused by Gram-positive bacteria. As one of the few new antibiotics to be released in the last decade (2003, Cubist Pharmaceuticals) [3], daptomycin highlights the ongoing potential for new microbial antibiotics.

Platensimycin (**3**) – First reported in 2006 from *Streptomyces platensis* by Merck [4], this class of antibiotics features a novel mode of action, targeting bacterial fatty acid biosynthesis (FabF/B). Addressing drug resistance is one of the major challenges in antibiotic development, and the discovery of platensimycin emphasises the importance of combining innovative bioassays featuring specialised molecular targets, with microbial biodiscovery.

Salinosporamides (**4**) – First reported in 2003 from a marine obligate *Salinispora tropica* [5], this structure class includes potent proteasome inhibitors which were fast-tracked into phase I human clinical trials against multiple myeloma [6]. As a key IP asset for Nereus Pharmaceuticals, the salinosporamides demonstrate the importance of marine microbes, and reassert the business opportunities that can flow from microbial biodiscovery.

Indolactam V (**5**) and *Stauprimide* (**6**) – These synthetic analogues of the *Streptomyces* metabolites, teleocidins and staurosporines, were reported in 2009 as the first small molecule modulators of embryonic stem cell (ESC) differentiation. Indolactam V directed hESCs into the pancreatic lineage [7], enhancing future prospects for new transplantation therapies for diabetes, while stauprimide primed ESCs for differentiation in response to intra- and extracellular signalling stimuli [8]. As the scientific community and society hold out the hope of stem cell-based therapies, microbial metabolites could play a critical role as developmental and differentiation reagents.

Cytosporone B (**7**) – Although a known fungal metabolite, cytosporone B was described in 2008 as the first natural ligand for the nuclear orphan receptor Nur77, rendering it a drug-led candidate for treatment of cancer and hypoglycaemia [9]. The structural simplicity of cytosporone B (compared to the likes of calicheamycin), and its relationship to an orphan receptor, underscores the importance of bioassay-directed microbial biodiscovery.

With microbial biodiscovery poised for a scientific and commercial renaissance, it is worthwhile reflecting on the contributions that Australia has made to this field in the past and might make in the future. As one of the few mega-biodiverse developed

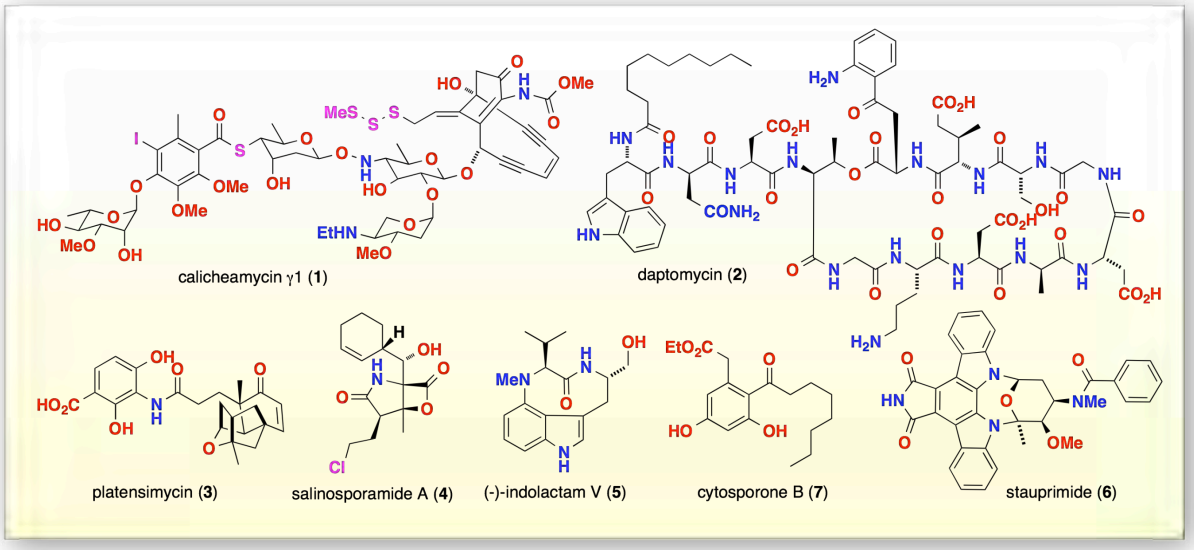

Figure 2. Selected microbial pharmacophores discovered/re-discovered over the last decade.

countries, with a long tradition of excellence in natural products chemistry, as well as microbiology, it comes as something of a disappointment to discover that of the mere 100 peer-reviewed scientific articles describing new metabolites from Australian microbes, almost half were authored offshore. It would appear that, with the exception of a few early visionaries, Australian science has yet to fully engage with microbial biodiscovery.

Not to be discouraged, over the last few years, with support from The University of Queensland and the Institute for Molecular Bioscience, and with the assistance of numerous talented students, staff and colleagues, we have attempted to redress this shortfall, to demonstrate by example the scientific and commercial potential of Australia-based microbial biodiscovery. Although still very much in a growth and learning phase, we have made significant progress and enjoyed many 'molecular' successes. Some noteworthy examples of these successes (Figure 3) include the antibacterial dipeptide aspergillazines (i.e. **8**) from *Aspergillus unilateralis* [10], the antibacterial and anticancer anthraquinone polyketide kibdelones (i.e. **9**) from a rare *Kibdelosporangium* sp. [11,12], and the rapamycin-like FKBP12 binding macrolide polyketide nocardiopsins (i.e. **10**) from a marine-derived *Nocardiopsis* sp. [13]. A schematic of our preferred microbial biodiscovery strategy is illustrated in Figure 4.

The observations presented above encourage the personal view that the future for Australian microbial biodiscovery is bright. This assessment is, of course, tempered by the challenges of attracting 'interdisciplinary' funding in a highly competitive and disciplinary-centric research climate and the closely related challenge to build and strengthen highly strategic and effective interdisciplinary networks and collaborations – with a particular emphasis on linkages between microbiology and chemistry.

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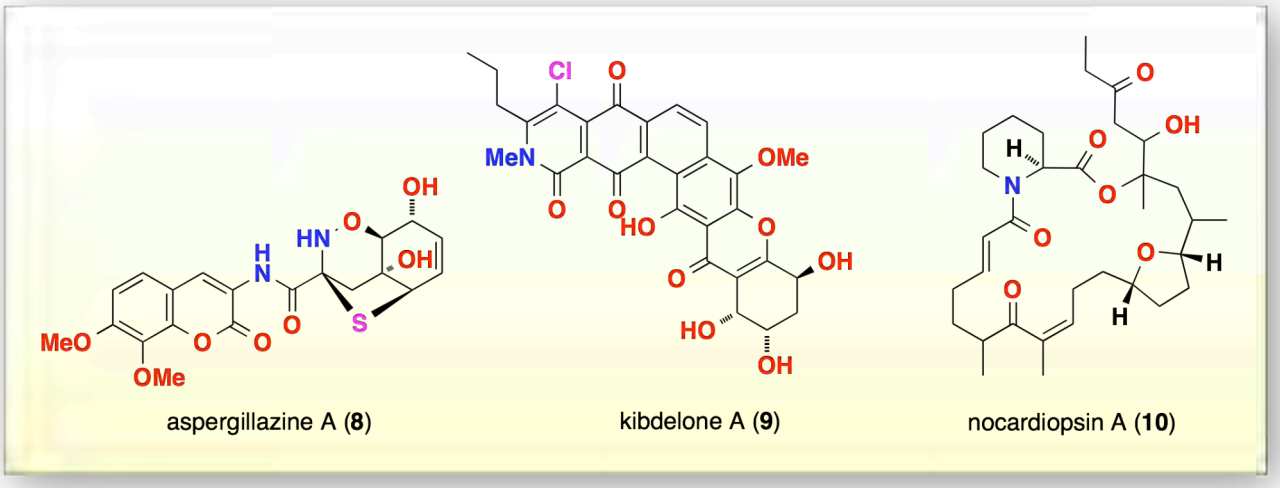

Figure 3. Australian microbe metabolites.

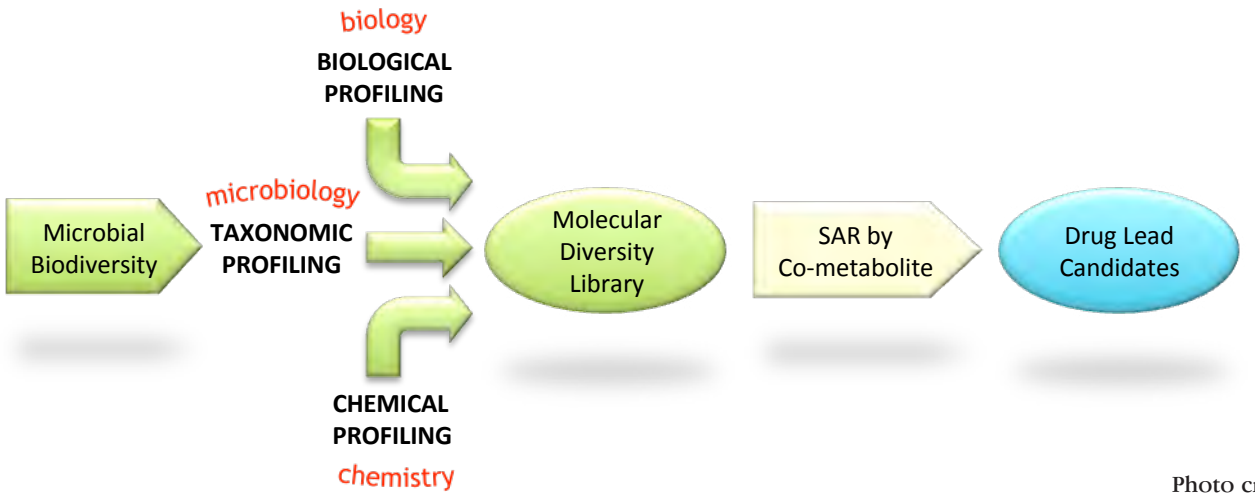

Figure 4. Schematic of a preferred microbial biodiscovery strategy.

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Biography

Rob Cappon's research group focuses on the detection, isolation, characterisation, identification and evaluation of novel bioactive metabolites from Australian marine and terrestrial biodiversity. These metabolites span all known biosynthetic structure classes including many molecules new to science, and their study requires the use of sophisticated chromatographic, spectroscopic and chemical technologies. Natural products uncovered during our investigations represent valuable new leads in the search for drugs with application in the fields of human and animal health and crop protection, have potential as molecular probes to better interrogate and understand living systems, and could find application as biological control agents.

Large-scale recombinant protein production and structure-based drug design capabilities at CSIRO

George Lovrecz, Geoff Dumsday & Tim Adams

Email George.Lovrecz@csiro.au
Geoff.Dumsday@csiro.au
Tim.Adams@csiro.au

CSIRO has opened its large-scale Recombinant Protein Production Facilities (RPPF) as part of the National Collaborative Research Infrastructure Scheme (NCRIS).

The new state-of-the-art facility is capable of the optimisation, scale-up, production and purification of recombinant proteins in large quantities: from hundreds of milligrams to kilograms to allow preclinical or even clinical trials.

CSIRO's facility is equipped with a range of cell culture and fermentation equipment to allow the use of a wide range of expression systems and host cells including mammalian, insect and microbial cells:

- Over 30 stirred tank reactors, ranging from 2 to 500L scale to allow rapid optimisation and large-scale protein production.
- Single-use bioreactors with working volumes of up to 25L.
- Roller bottle apparatus, spinner and shaker flasks suitable for scale-up and process development work.
- A wide variety of analytical equipment to follow cell growth, metabolism and characterisation of proteins.
- Downstream and purification equipment suitable for processing large-scale batches of microbial and mammalian cell cultures.

Recent projects have included the production of:

- Monoclonal antibodies.
- Receptor signalling and cytokine proteins.
- Process development and production of bioremediation enzymes for field trials.
- Production of small molecules via microbial biotransformation.
- Process development and production of structural proteins to demonstrate proof of manufacture and for materials testing.
- Process development and production of malaria vaccine candidates.
- A wide range of other bacterial and insect cell proteins.

The RPPF is the largest research laboratory in Australia open for collaborative or fee for service projects providing access to process development, optimisation and protein production at best-practice standards.

The facility is supported by the expert capabilities of various other molecular and cell biology groups to allow cell line development and protein chemistry for rapid purification and characterisation of proteins. Figure 2 provides further details. There is also a