Harnessing Nature's wisdom

Turning to Nature for inspiration and avoiding her follies

For most of human history, Nature provided everything that humans needed: food, clothing, tools and medicines. The invention of metallurgy, engineering and eventually modern chemistry increasingly replaced natural products with man-made commodities: enhanced tools and machinery, clothes from synthetic fibres, processed food enriched with nutraceuticals and flavours, and pharmaceuticals. Yet, the pharmaceutical industry was also the first to return to Nature for both inspiration and to identify possible drug candidates. But this renewed interest in natural products reaches beyond medicine to other industries, which seek to exploit molecules and structures that have been perfected during millennia of evolutionary pressure to fulfil their specific tasks.

Notable examples of natural products in medicine that have become important drugs include penicillin from the mould *Penicillium notatum*, morphine from the opium poppy and the anticancer drug taxol from the Pacific yew tree. Indeed, half of all therapeutic drugs, and an even greater proportion of narcotics, are based on natural compounds. Since the beginning of the pharmaceutical industry in the 1940s and 1950s, companies have been looking at Nature for new drugs, but research on natural products temporarily declined in the 1990s. One reason for this downturn was the continuing difficulty of extracting or synthesizing natural products in industrial quantities and at an acceptable cost. Another reason was the advent of combinatorial chemistry, which allows the synthesis of an enormous number of compounds for drug screening. A molecule of interest might have, for example, three sites of variation, each of which could have 10 different forms. This would generate 1,000 variants ($10 \times 10 \times 10$), which could be created to produce libraries of related but subtly distinct compounds. Combinatorial chemistry was increasingly combined with *in silico* methods to spread the net even further, and superficially seemed to represent a great leap forward in drug discovery.

Yet, combinatorial chemistry has proved to be a false lead. It has failed partly on

account of scale; although it created a large number of compounds, these still represent only a tiny fraction of the total possible number of chemical and geometrical permutations. Moreover, it also turned out to be counter-productive for drug discovery to randomly screen compound libraries across all possible combinations of structure and atomic constituent. "It is clear today that biological activity is not randomly scattered in diversity space, and that there are islands of activity," commented Nicolas Winssinger, Professor of Chemistry at the Université Louis Pasteur in Strasbourg, France. Natural products therefore can help researchers to identify these areas of activity, Winssinger added and suggested that the techniques of combinatorial chemistry can then be applied more effectively to explore within these islands. In a sense, natural products represent "nature's own high throughput screening through evolution", as Ian Paterson put it, a synthetic biology specialist at Cambridge University, UK.

The insight that natural compounds are useful beacons to seek and explore molecules that interact with a particular target is further stimulated by genomics and proteomics projects, according to Kamal Kumar, group leader for the synthesis of natural-product-inspired compound collections at the Max Planck Institute of Molecular Physiology in Dortmund, Germany. "Keep in mind the continuously increasing druggable targets from genomics and proteomics research," said Kumar. "To identify their functions we need suitable ligands for these new targets and natural product based compounds can do that job much better."

Such natural products and synthetic compounds have a wide range of potential applications, and are not confined to anticancer and antibiotic drugs. As Winssinger pointed out, most natural products are derived from microorganisms and are cytotoxic, having evolved primarily for chemical warfare. But in some ways their belligerent role is just an outward manifestation; natural products actually comprise highly conserved molecules that can operate within

various pathways and organisms, and that are optimized for fulfilling specific tasks.

"Natural products can be regarded as prevalidated by biology because they were made by enzymes and because their function is based on binding to proteins," said Herbert Waldmann, Professor of Chemistry at the Max Planck Institute of Molecular Physiology. "[T]hey have the ability to interact with multiple proteins. Also, when exerting their mode of action, they need to reach their targets (mostly intracellular proteins), thus they must have certain important pharmacological parameters, such as membrane permeability."

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Novel compounds derived from natural products are therefore more likely to retain this compatibility with biology, said Waldmann, and should provide a fertile source for drug discovery. But it also highlights a major problem facing natural products, and the reason why these have not made much greater progress in the fight against cancer and infectious disease; although there is no problem identifying potent candidates, it is difficult to handle the sometimes devastating side effects. "We haven't figured out how to put a target molecule that might interact with some enzyme into something as complex as the human body, and still get that selectivity while not interacting with other things," said Scott Snyder, whose group at Columbia University, New York, NY, USA, specializes in the synthesis of natural products.

Kumar went even further by suggesting that, given their complexity, natural products are in fact prone to having adverse or unintended effects. "The pharmaceutical industry is often concerned about the toxicity of natural products which could be because of their great structural complexity (because of which they can bind to multiple proteins)," said Kumar. One example

is the powerful antitumour compound roseophilin, derived from the bacteria *Streptomyces griseovirides*, which is unfortunately too toxic to be used as a potential therapeutic (Fürstner *et al*, 2004).

Furthermore, natural products are unlikely to interact with just one specific target such as a particular pathogen, commented Jeremy Robertson, whose group at Oxford University, UK, also works on the synthesis of natural products. "Many natural products occur in their host because they confer some selective advantage which may or may not bear relation to the therapeutic activity that we value," he said. Thus, the challenge is to identify and isolate the binding sites or components of a natural product that have the therapeutic effect, while eliminating the parts that cause toxicity or other side effects. Robertson is attempting to do this with roseophilin by studying its stereochemistry. Although the molecule's exact mechanism of action still remains unknown, Robertson speculates that the mechanism might involve interchelation of DNA to inhibit cell replication or alkylation of the DNA bases, which has a similar effect. Indeed, the big question is how to sort out the 'good and bad bits'

of natural products, especially when the mechanism is not perfectly understood. "That is the main purpose of our research, to improve upon natural products which are good starting points but need modifications to suit our purpose," said Kumar. "We call it natural product inspired synthesis."

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This might involve using the characteristic protein scaffold or the core structure of a natural compound and changing surrounding components. In some cases, the core structure itself might be changed slightly, using it as an inspiration for further engineering. Kumar's group uses both forward and reverse genetics to identify proteins or active sites with a particular function, even if the mechanism of action is not known. Working this way to identify crucial components, rather than attempting to synthesize the whole natural product, is more likely to yield useful compounds, said Kumar. "As compared to a total synthesis of a known natural product, this approach is more

efficient and practical. This has been successfully done in many cases mostly for chemical biology and chemogenomics studies, and it seems promising enough for the industry to take up for the drug discovery process."

It is the initial inspiration that remains one of the most exciting aspects of this work, commented Robertson, both in terms of design and synthesis. "Nature opens our eyes to what's possible," he said. "Some of the most esoteric-looking structures, that wouldn't be dreamt-up in a chemist's mind, are natural products. At the same time the publication of a new natural product with a unique structure poses new chemical challenges that inspire synthetic chemists to design ever more powerful synthetic methodology."

Examples of the value of natural products are potential new antibiotic compounds against hardy pathogens such as MRSA (multi-drug resistant *Staphylococcus aureus*) that are difficult to eradicate from hospitals and nursing homes. Two promising candidates, platensimycin and platensin, are now being studied by a group at the Scripps Research Institute (La Jolla, CA, USA) under Kyriacos Costa Nicolaou, the Cypriot-born chemist who developed a route to synthesize taxol in 1994, thus avoiding reliance on

the Pacific yew tree. Both compounds are derived from the bacterium *Streptomyces platensis* and are examples of harnessing the products of chemical warfare among micro-organisms. Both block enzymes involved in the condensation of fatty acids, which are crucial constituents of the cells' membranes; their absence renders bacteria much more susceptible to the host's immune system. Both compounds also rely on a broad-spectrum mechanism rather than a specific target on the bacteria surface, which might make it harder for bacteria to develop resistance. In fact they also have potential against other classes of pathogens, especially viruses.

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But to be effective against MRSA in humans they must reach their target efficiently and not cause side effects in the host. It is attention to these important issues that distinguishes research on naturally derived drugs from the field of herbal medicine, which, by definition, also involves natural products. "The difference is that chemists are talking about pure active ingredients, precisely defined at the molecular level, as opposed to crude, multi-component mixtures of compounds contained in herbal medicines," said Nicolaou.

Research on natural products is by no means confined to medicinal applications, even if these predominate because they attract most funding. Spider silk, or gossamer, for example, has been inspiring material chemists for a long time owing to its unique combination of strength and resilience. Although not quite as strong as the toughest man-made fibres, gossamer has remarkable elasticity, which allows spiders' webs to absorb the impact of airborne prey; some varieties are able to elongate to three times their original length without breaking. This, in combination with its light weight and environmentally friendly composition and assembly, have prompted great efforts to emulate the functional properties in artificial fibres. The first step was to identify the crucial processes in assembly (Jin & Kaplan, 2003), and the next challenge is to link structure with function and to identify

just what makes spider silk so strong and yet elastic. This is still not fully resolved, but it seems that both the alignment and surface properties of the constituent polymers have a crucial role (Kojic *et al*, 2006). The overall structure resembles tangled and oily spaghetti, which allows the strands to slide past each other and deform in response to stress.

The other important challenge lies in assembly: the silk emerges as a liquid and the protein fibres crystallize in an irreversible reaction, similar to the process whereby egg white transforms from a clear, sticky solution into a white, opaque rubbery base under heating. Unravelling this spinning process also remains an open question, although some progress has been made after a team of UK and Chinese scientists discovered that temperature and mechanical processes involved in the spinning are at least as important as the actual protein constituents (Shao *et al*, 2006). Potential applications of synthetic spider silk include bullet-proof vests, ropes, nets, parachutes, surgical thread—retaining the biodegradability of spider silk—or artificial tendons—where biodegradability is obviously not wanted.

Natural products can also elucidate new ways of accomplishing specific tasks or functions. One example is the protection of biological tissue against cold by natural antifreeze proteins. In many cases, nature protects against tissue or cellular damage caused by the formation of ice crystals by recruiting salts or producing proteins to lower the ambient freezing point. But this is not always sufficient, particularly within animals that cannot tolerate high ionic concentrations. In such cases, organisms have evolved a more subtle method by inhibiting the growth of ice crystals when the ambient temperature drops below the freezing point of the organism's internal fluids.

The discovery of this mechanism followed the observation that some insects are able to survive cold winters and withstand temperatures far lower than the freezing point of their fluids. One such insect that has been widely studied is the spruce budworm, which occurs as a conifer parasite in western North America. The mechanism of its antifreeze proteins has now been elucidated by using fluorescence microscopy (Pertaya *et al*, 2008). It revealed that these proteins inhibit the growth of ice crystals by accumulating along the corners of the prism of crystal growth. The result is that ice crystals are truncated and too small to damage the insects' tissues.

These proteins highlight a remarkable adaptation for survival in cold climates, which could be used elsewhere, for example, by modifying crops to confer greater protection against frost. Some plants such as carrots already have such proteins to protect against frost damage to their roots, even if they are not as effective as the budworm's. Such plants could also potentially be adapted to produce antifreeze proteins on a commercial basis for various applications, for example, to reduce ice crystal damage to human tissue stored in biobanks or organs for transplantation.

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The example of antifreeze proteins also shows that, although pharmacological applications of natural products have attracted the greatest interest so far, there is also a vast potential for non-medicinal uses. Yet, the renewed interest in natural products is not only driven by looking at nature as a source of new materials or compounds; the powerful tools of modern science allow a better understanding of why natural products evolved and how they work, and thus allow scientists to build on or draw inspiration from these. Indeed, for many a medical or other problem, it is not necessary to design completely new solutions from scratch: a close look at nature and some intelligent tinkering with its products might suffice.

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doi:10.1038/embor.2008.160