EXPLOITING THE FUNGI: NOVEL LEADS TO NEW MEDICINES

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This article discusses how we are looking at natural sources, using fungi in particular, to give us novel lead molecules which could supply some clues for the generation of new medicines.

Since his earliest days man has turned to Nature for medicines. In more recent times, for the fungi, the discovery of penicillin was the watershed. There was the sudden realisation that fungi, and other microorganisms, could offer us a tremendous source of pharmaceutically useful molecules. Once the significance of penicillin was realised, there was the need to produce it in large quantities and now it is produced by the tonne in 200,000 litre fermenters. Different penicillins are found naturally; those used today have been chemically modified to give a better spectrum of activity and are termed semi-synthetic penicillins.

From the forties onwards there was an explosion in the discovery of compounds from microbes, with a wide variety of biological activities. Another important family of antibiotics was soon found, that were named cephalosporins from the fungal genus *Cephalosporium*. The original source of the cephalosporins was less romantic than penicillin; no spore floating through a window, but the fungus coming from a sewage outfall, illustrating the fact that useful fungi may be found anywhere!

Why are such substances produced in nature? Antibiotics may give an organism a competitive advantage. For other molecules the possible roles in the producing organism are unclear, yet many systems in cells are common across the evolutionary scale; enzymes, proteins and various biological systems in the microbial cell may be very similar to those in the human cell. Cyclosporin was probably not developed in the fungus *Tolypocladium* in order to suppress the human immune system, but the mechanism by which cyclosporin works in the human is likely to have something in common with the mechanism by which the compound acts within the fungus itself.

There are various advantages and disadvantages in looking to nature for leads to new medicines. It is highly unlikely that even today synthetic chemists would have dreamed of the structures of compounds produced by fungi. Nature has generated molecules very unlikely to have come from synthetic origins. The size, surface area and functionality of such molecules are particularly suitable for interactions with biological systems. The molecules are there for a purpose, even if we do not know what that purpose is, and it has been said that they are solutions to challenges which have been confronted and overcome during molecular evolution.

There is unrivalled chemical diversity. Families of compounds are often found: when we find an active molecule from a fungus there is often a family of closely-related molecules and by studying the activities of these we can build up structure/activity relationships and pose the question 'what is it about the particular features of the molecule that give it its activity'? We can manipulate the biosynthesis; we can grow fungi in particular conditions to alter a molecule; we can scale up a fermentation and so gain large quantities of a compound.

There are some perceived disadvantages where molecules are complex and difficult to synthesise. Industry's preference is for chemical synthesis, so we look at samples from nature to provide leads which medicinal chemists can then develop. However, although penicillin can be made synthetically, it would be prohibitively expensive to do so and thus it is still made by fermentation.

If we look at the top 20 selling prescription medicines in 1995 (Table 1) we see three closely related compounds: pravastatin, simvastatin and lovastatin, all derived from a molecule from *Aspergillus terreus*, mevinolin. These 'statins' are used to reduce cholesterol levels in the body (as discussed later). There are a further three compounds derived from fungi: amoxicillin, a semi-synthetic penicillin, which is sold in combi-

Table 1 Top twenty selling pharmaceuticals, worldwide, in 1995

Compound	*Mode of action*	*Sales $m*
Ranitidine	H2-antagonist	3914
Omeprazole	proton pump inhibitor	2539
Enalapril	ACE inhibitor	2220
Nifedipine	calcium antagonist	2191
Pravastatin	hypolipidaemic	1980
Fluoxetine	antidepressant	1790
Simvastatin	hypolipidaemic	1615
Lovastatin	hypolipidaemic	1345
Acyclovir	antiviral	1313
Ciprofloxacin	antibiotic	1279
Amoxicillin +clavulanate	antibiotic	1176
Captopril	ACE inhibitor	1110
Cyclosporin	immunosuppressant	993
Diclofenac	NSAI	938
Ceftriaxone	antibiotic	909
Famotidine	H2-antagonist	850
Methyltestosterone	hormone	840
Salbutamol	b-2 agonist	825
Fluconazole	antifungal	800
lisinopril	ACE inhibitor	772

nation with another compound; cyclosporin - an immunosuppressive agent, and ceftriaxone - a cephalosporin antibiotic. In this top twenty, six compounds are of fungal origin.

Are there fungi left to look at? Estimates of some 1.5 million fungi have been made; certainly less than 0.1 million have been characterised to date. Fungi are ubiquitous - a single gram of soil may contain a million fungi. If we obtain soil and plant material from a variety of ecological niches we may isolate a wide variety of fungi.

Individual fungi are isolated from nature into pure culture, often on media provided with selective chemical inhibitors to prevent the fast-growing fungi becoming dominant. Colonies arise from single spores invisible to the naked eye. Isolation of fungi is still a naive activity since many fungi may not grow on the nutrients supplied. This stage of the work calls for an experienced mycologist to examine the colonies and avoid too much duplication - one or two colonies of each type are selected and each is grown on a separate plate in isolation. We end up with a collection of individual fungi. These are put into long term storage, into dilute glycerol suspensions stored in liquid nitrogen vapour, and are retrieved as necessary for screening. Fungi from soil are mostly microfungi, but we are also interested in macrofungi which produce visible fruiting bodies. We may either culture the fungus and grow it as we grow a microfungus, or use fruiting bodies. Plant material is also an important source. Pieces of root, for example, are surface-sterilised and plated out: here we do not want organisms from outside the root, but endophytes growing within the plant tissue.

It is critically important how our fungi are grown and harvested. We have to consider the nutrients that we use, the acidity or alkalinity of the growth medium, the physical conditions of the medium, the length of incubation: growth may be rapid, but compounds may be produced later on.

For screening we place samples of the extracts into microtitre plates, each consisting of 96 wells which may be considered as 96 miniature test tubes. We put a small sample of fungal extract (ca 10 microlitres) into each well and add a test system by which we can determine whether there might be a lead molecule in the fungal extract. When we detect activity in a fungal extract we must isolate and characterise the active component.

How does this screening work? We aim to look at processes involved in human diseases. In arthritis, asthma and cancer, we see a situation where the chemistry within cells has become abnormal and the change has caused the diseased state. We attempt to use drugs to rectify change and put the cell chemistry back to normal. We can only do that if we understand what has changed, and then identify a screening target which may be amenable to therapeutic intervention. Once we have established a screen, we subject it to samples of wide chemical diversity which might interact with our target and provide us with a lead molecule. Chemists can then modify the molecule to give us a possible drug candidate.

There are many examples of screening which involve enzyme targets, for diseases where the chemistry of the cells has changed. Very often this is associated with an elevated level of a particular enzyme. If the enzyme is inhibited, disease may be remedied. For example, high cholesterol levels are deemed a risk factor in

heart disease. In individuals with high cholesterol levels, diet may be a factor, but more often the body itself is producing the high level of cholesterol. If we study the chemical pathway by which cholesterol is made in the body, we can look for substances which would inhibit one of the steps in that pathway and reduce cholesterol production. Fig 1 shows that cholesterol is formed from a long series of steps, each catalysed by an enzyme.

We know that HMG CoA reductase is an enzyme involved in synthesis of cholesterol in the body. Can we find an inhibitor of this enzyme? We see from the top twenty pharmaceuticals (Table 1) that the three statins are all effective inhibitors. The sequence of events is: What is the problem, what is the biochemistry, what target might we use as a screen, can we find inhibitors, could an inhibitor become a drug? In this case, a resounding yes. Looking again at the biosynthetic pathway, we might inhibit another enzyme lower down the pathway, e.g. squalene synthase. Statins are effective in lowering cholesterol but they have a drawback in that they also reduce other intermediates in the pathway which have other functions.

Another approach to the cholesterol problem is to look at enzymes involved in deposition of cholesterol in the arterial walls; not to be concerned so much about production of cholesterol but to stop it being bound onto arterial walls causing atherosclerotic plaques. ACAT is an enzyme involved in that mechanism. We have pulled out from natural sources a number of interesting molecules including those from fungi which have striking similarity to cholesterol. ACAT takes cholesterol and converts it into an ester form. These molecules are very similar to cholesterol. We can use the analogy of the enzyme as a lock and the substrate as the key. Fungal molecules similar to the natural key could bind into the enzyme and stop it conducting its usual mechanism.

What of the future? Screening is a key part of our research strategy because there are many unmet therapeutic needs, either with no therapy or poor therapy. The identification of new screening targets continues, and will increase further as the human genome project uncovers genes involved in disease. Advances in robotics, automation and information technology present us with the opportunity of testing large numbers of samples in the search for new lead molecules. There is no doubt that the fungi will continue to offer us a tremendous source of the novel molecules we seek.

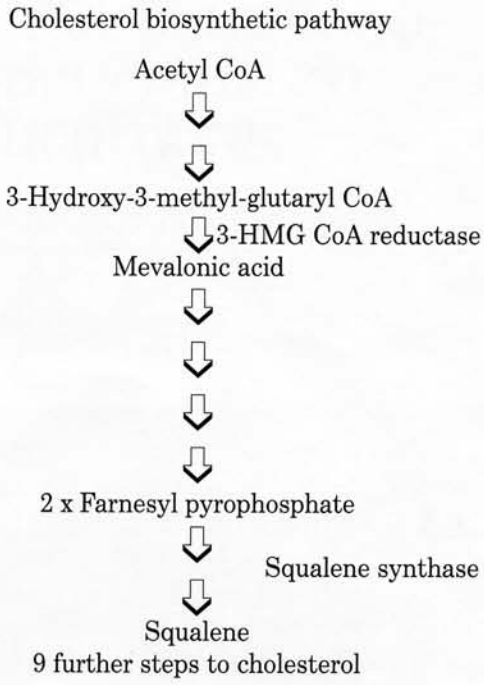

Fig 1 Simplified pathways in cholesterol synthesis.

Editor's note: This article is based on a public lecture by the author, given as one of a series of Popular Lectures at the Fungus 100 Exhibition, September 1996. Others in this series will be published in forthcoming issues.