

IN SEARCH OF NEW LEADS

by Hugo Kubinyi

Pharmaceutical industry is short of new drugs. Whereas in past decades about 50-60 new drugs (new chemical entities, NCEs) were approved every year and introduced into therapy, this number declined significantly in the last few years, reaching its historical low in the year 2000 with 27 NCE's, 2001 with 24 NCEs, and 2002 with only 18 NCEs approved by the FDA. Correspondingly, research costs for a new drug are estimated to be \$500-900 million dollars.

There are several reasons for the decline in the number of new drugs. The two most important ones seem to be an already achieved high therapeutic standard in many indications, so research is now focusing on chronic degenerative diseases; as well as enhanced regulatory requirements. However, the current situation reflects also a shortage of new lead structures that can be optimised into therapeutically useful drugs. This overview examines different strategies in the search for new leads.

Natural products as traditional sources of lead structures

Natural products have always been the richest source of drugs and lead structures. About half of our drugs are still natural products, derivatives, or analogues of natural products such as foxglove, opium, quinine and salicylic acid. Whereas, in the past, plant products played a predominant role and microorganisms were only investigated as producers of antibiotics, nowadays several important classes of drugs are extracted or derived from microorganisms.

Since 1928, when Sir Alexander Fleming discovered the lysis of bacteria by a secretion product of a *Penicillium* strain, microorganisms have been a rich source of antibiotics. The original penicillin structure has been optimised, step by step, to bioavailable analogues, to broad spectrum antibiotics, and finally to lactamase-resistant derivatives. In addition to penicillin, the cephalosporins, tetracyclines, chloramphenicol, streptomycin, rifampicin, vancomycin, etc., turned out to be valuable lead structures or antibiotic drugs themselves. But not only antibiotics resulted from microorganisms, also cardiovascular drugs and the

hallucinogenic lysergic acid diethylamide (LSD) from ergot (*Secale cornutum*), the immunosuppressants cyclosporin A and tacrolimus, the antitumour principle epothilone, and the important group of cholesterol biosynthesis-blocking statins. Also the anticoagulant coumarins, like phenprocoumon and warfarin, were derived from dicoumarol, a microbial product first isolated from rotten hay.

Serendipitous drug discoveries

Some of the very first drugs were discovered by serendipity, over 150 years ago. The use of nitrous oxide and ether as narcotic gases in surgery resulted from the observation that those people who inhaled these chemicals in fun parties did not experience any pain after being injured. The vasodilatory activity of amyl nitrite and nitroglycerin was also discovered by accident; chemists working with these organic nitrites experienced strong headache after inhaling or ingesting minor amounts. Phenolphthalein was used to colour cheap wines but in a heroic self-experiment, a pharmacologist experienced its drastic diarrhoeal activity. A secretary fell asleep for about 20 hours after the first human application of clonidine, which was supposed to be a nasal congestant but turned out to be a strong antihypertensive drug. The stories of the serendipitous discoveries of penicillin, LSD, warfarin, all the major artificial sweeteners and the first tranquilliser, chlordiazepoxide, are well known.

A closer inspection of drug discovery stories shows that serendipity and sagacity played an important role in many cases. Fleming might have discarded his spoiled bacteria culture and Sternbach might have neglected the crystals of chlordiazepoxide when he cleaned up his laboratory. But they didn't because they were experienced investigators, "Chance only favours the prepared mind" as Louis Pasteur once said.

Rational approaches – the golden age of drug research

Besides natural products from plants, endogenous neurotransmitters and steroid hormones have been the richest source of new drugs. From the elucidation of the biochemical mechanisms underlying the transmission of nerve impulses and the deeper understanding of hormone effects, a large number of therapeutically useful drugs resulted, not only receptor agonists but also antagonists. This phase of drug research may be considered as its golden age. Nearly every modification of dopamine, serotonin, histamine, or acetylcholine, using the modification strategies of classical medicinal chemistry, resulted in a compound with modified

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activity and selectivity, most often in a drug candidate. The very first H1-antihistaminic drug, diphenhydramine, was synthesised in the 1940s. Then by serendipity, it was discovered that dimenhydrinate, the complex of 8-chlorotheophylline with diphenhydramine, is an efficient drug against travel sickness; its "clinical trial" occurred in 1947, on the voyage of the "General Ballou" from New York to Bremerhaven. Diphenhydramine became such a financial success that the royalties for its inventor exceeded the income of the president of the company Parke Davis, which distributed the drug; later this inventor became its Director of Research.

Similar success stories can be told about the steroid hormones and their more selective synthetic analogues.

"Me too" research

Copying existing drugs, with only minor chemical variations, is designated as "me too" research. Whereas the marketing of analogues without major therapeutic advantages does not promise any benefit, many examples demonstrate that later analogues show indeed major advantages, like the bioavailable, broad-spectrum, and lactamase-resistant penicillins (see above), the diuretic and antidiabetic sulfonamides that were derived from antibacterial sulfonamides (see later section), polar H1 antihistaminics without sedative side effects, or β 1-specific antagonists as well as partial agonists, with and without α 1-antagonistic activity, as compared to the original nonspecific betablockers. "Me too" is now no longer the goal of pharmaceutical industry, but "me better", "me first" or even "me only".

Peptides to peptidomimetics

Many substrates of enzymes, e.g. angiotensinogen, angiotensin, fibrinogen (as a precursor of fibrin), HIV GAG and GAG-POL proteins (the precursor proteins of HIV protease and other HIV proteins), and many receptor ligands, are either peptides or proteins. In contrast to protein-protein interactions in signalling chains, the interaction of these ligands with their target is often mediated by only a few amino acid side chains.

Peptides can easily be synthesised in large number – even millions or billions of different analogues are no problem, if parallel synthesis is used to produce mixtures of analogues. Correspondingly high-affinity substrates or ligands can be readily discovered. However, the next step, the chemical conversion of such a peptide lead into a non-peptidic ("peptidomimetic"), bioavailable drug is far from simple and cannot be considered to be a straightforward, generally applicable strategy.

The optimisation of drug side effects

Most drugs show, in addition to their main mechanism of action, some side effects which have often paved the

way to applications in a different indication. A very early example was the organomercurials (now obsolete), which were originally used for the treatment of syphilis but turned out to act as diuretics. Alternative drugs that are still used today resulted from the optimisation of the diuretic side effects of antibacterial sulfonamides. After the observation of severe hypoglycaemic effects in patients, caused by another antibacterial sulfonamide, antidiabetic drugs were developed from these leads.

A prominent example of the "use" of a drug side effect for therapy, from our time, should be mentioned. The first drug for the treatment of male sexual disorder, sildenafil, resulted from the development of antiallergic, antihypertensive and antianginal drug candidates; in a tolerance study in man, a surprising side effect of strengthening penile erections showed up, which finally led to the development of sildenafil in this therapeutic direction.

Prodrugs

Converting drug candidates with good *in vitro* properties but insufficient *in vivo* properties, e.g. poor bioavailability, into prodrugs, is a general strategy in lead optimisation. Very first examples have been acetylsalicylic acid (however, in this case producing a completely new mechanism of action, see above) and heroin, the diacetyl derivative of morphine. As prodrugs have been extensively reviewed, only one example shall be mentioned here.

The anti-ulcer drug, omeprazole, was not developed as a prodrug but it turned out to be a drug with probably the best organ selectivity. In an acid-resistant formulation it passes the stomach, is absorbed in the intestine and is distributed all over the body. In the acid-producing cells of the stomach, and only there, it is activated by an acid-catalysed rearrangement to irreversibly react with and inhibit H^+/K^+ -ATPase, the so-called proton pump.

Biological activities of enantiomers – the chiral switch

In the past, chiral drugs were developed as racemates or as diastereomeric mixtures, if two or more chiral centres were present. Only about 20 years ago, the pharmacologist Ariëns criticised racemates as compounds "including 50% impurity" and so made the pharmaceutical industry aware of the problem that a drug and its mirror image might have significantly different biological activities. Indeed, some chiral barbiturates are sedative in their active form, whereas their enantiomers cause convulsions; with some synthetic morphine analogues, the one enantiomer is a strong analgesic, whereas the other one is an antitussive drug. In the last decade, companies have extended the

lifetime of their chiral drugs, if originally marketed as a racemate, by a so-called "chiral switch" i.e. by marketing the biologically active enantiomer instead of the racemate. Examples of this strategy are dexfenfluramine (withdrawn 1997), levofloxacin, levobupivacaine, esomeprazole and levocetirizine.

Rescuing poor leads – the metabolic switch

Sometimes, leads have such poor properties that neither classical optimisation nor a prodrug derivative can help. Nevertheless, such compounds can be "rescued", either by understanding the biochemical mechanisms, by selecting a metabolic precursor, or by selecting an active metabolite of an otherwise inactive or toxic drug. Two examples, dopamine and phenacetin illustrate some of these approaches.

Parkinson's disease results from a lack of dopamine in certain brain areas. A substitution by oral application of dopamine, is impossible due to its poor bioavailability and insufficient blood-brain barrier penetration. L-Dopa, the metabolic precursor in its biosynthesis, offers a good chance because it is actively transported, in absorption as well as through the blood brain barrier. However, peripheral side effects, like increase of heart rate and blood pressure, and short biological half-life limit its therapeutic value. Both are compensated by co-application of a polar dopa decarboxylase inhibitor, which acts only in the periphery, and a centrally active monoamine oxidase inhibitor, resulting in a unique success of rational combination therapy.

Phenacetin has been used for decades as a mild analgesic and antipyretic principle before liver toxicity and nephrotoxicity after chronic abuse caused its withdrawal from the market. Its active metabolite paracetamol does not form these toxic metabolites and has replaced phenacetin.

Screening and high-throughput screening (HTS)

There is no question that screening has contributed to the discovery of many valuable leads. However, companies are now aware that the original concept to throw their compound collections, any commercially available compounds, or combinatorial libraries (see next section) on many new biological targets did not deliver to the expected extent. Limited solubility, deposition after dilution with buffer, compound decomposition in the storage solution, etc., produce legions of false negatives and false positives. In many cases, re-testing does not confirm any primary hits; in other cases, re-testing of analogues that are similar to confirmed hits uncovers their activity, although they were initially found to be inactive.

Another important question arises: is target focus really the best strategy or were whole animal experiments better suited for the search of new leads? There is no way back to animals as screening models but one has to consider that several drugs, e.g. antidepressants and neuroleptics, exert a broad spectrum of different activities.


Combinatorial chemistry

Even more disappointing than HTS results with historical compound collections was the poor success rate of combinatorial libraries, especially in the early years. Huge libraries of ill-defined mixtures of most often lipophilic and too large compounds were tested, without any positive result. Only after the introduction of the virtual screening techniques, people became aware of the importance of certain drug properties, like appropriate molecular weight and balanced lipophilicity.

In the meantime, combinatorial chemistry developed into automated parallel synthesis of much smaller libraries of single and pure (or purified) compounds of biological interest. Its main application is nowadays not so much in lead structure search but in lead validation and in the early phases of lead optimisation. A convincing example of the proper application of combinatorial chemistry in early lead profiling is, e.g. the discovery of nanomolar somatostatin receptor subtype-selective ligands in several libraries, with up to 350,000 members per library.

Virtual screening

In classical medicinal chemistry, drug discovery always started from a lead. In this approach, the often-quoted ratio of one drug per 10,000 new molecules was a realistic estimate. In our time, with combinatorial chemistry and high-throughput screening, this ratio changed to hundred thousands or even millions of test compounds for a new drug. Relatively often, no hits at all are discovered in HTS and the corresponding target is then called a "non-druggable" target. But even in positive cases, not every screening hit can be confirmed and later validated by the synthesis of close analogues and not all validated hits are suited as leads, according to their physicochemical properties.

Virtual screening is a toolbox of methods to select appropriate candidates, in order to enrich compound collections and combinatorial libraries with promising candidates. As the input of these techniques are only chemical structures and calculated properties of the compounds, virtual screening can also be applied to virtual libraries of almost any size. 

Structure-based ligand design

The large number of protein 3D structures that is available from the Brookhaven Protein Database (22,823 entries; August, 2003) enables scientists to perform, in principle, a de novo construction of ligands that fit a certain binding site, in shape and in all other properties.

The very first drug, which resulted from structure-based design, was Captopril which was modelled from the 3D-structure of an inhibitor complex of the related enzyme carboxypeptidase. Other drugs followed, eg the HIV protease inhibitors nelfinavir and amprenavir; many more are in clinical development. Structure-based design is now a most important technique in cases, where the target 3D structure is known or accessible.

Computer-aided ligand design

Molecular modelling started about 25 years ago, with the presentation and real time rotation of a molecule in front of a computer screen. Within a short time it developed into a highly valuable tool in drug design, especially in helping the medicinal chemist to establish and evaluate working hypotheses on structure-activity relationships.

Goodford's computer program GRID inspects the surface of a protein, especially its binding site, with different chemical probes, to search for "hot spots" where a certain functionality of a ligand should favourably interact. The most impressive example was the application of GRID to the viral enzyme neuraminidase: introduction of a guanidinium group into this lead increased affinity by about 4 orders of magnitude, leading to the influenza drug zanamivir.

Fragment-based ligand design

The chance of a ligand to bind to a protein depends on its complexity. Smaller ligands have more possibilities to be accommodated, which was considered in the first small ligand library of the program LUDI as well as in the MCSS (multiple copy simultaneous search) docking program, which uses functional groups and small molecules to search for an ensemble of favorable locations within the binding site.

Combinatorial ligand design

The concept of fragment-based ligand design has been extended to combinatorial techniques, where a multitude of ligands is tested in the search for new leads. The advantage of this approach is its independence on the development of a specific screening method for a new protein; certain problems may arise from the restricted mobility and accessibility of the ligands. The dynamic assembly of ligands

generates ligands from fragments that can reversibly react with each other in the presence of a protein. Ligands that fit the binding site are preferentially formed and afterwards trapped by a reaction that freezes the equilibrium; the application of this principle has been illustrated by the generation of carbonic anhydrase and neuraminidase inhibitors.

In addition to these experimental techniques, there are several computer-assisted techniques for the combinatorial combination of fragments to new leads.

Summary and Conclusions

If one considers the broad range of approaches used to arrive at new leads, it is surprising that lead search indeed poses a problem. However, traditional sources, like plant products, microbial metabolites, endogenous neurotransmitters and hormones, are to some extent "exhausted". High-throughput screening (HTS) and combinatorial chemistry have not delivered to the expected extent. Virtual screening and fragment-based approaches have just started but they seem to be the most powerful techniques for the near future; compound collections and virtual libraries can be enriched with promising candidates which can be tested with greater care than usually applied in routine HTS runs. In the very end, the integration of protein crystallography, NMR techniques, and virtual screening will significantly enhance the pace of the discovery process and the quality of compounds selected for further development.

In their search for new leads, as well as in lead optimisation, medicinal chemists always followed the similarity principle, that similar compounds should exert similar biological activities. Despite many exceptions to this general experience, drug research focuses now very often on target families. The term "chemogenomics" has been coined for the investigation of certain compound classes in target families, like the G protein-coupled receptors (GPCR), the serine proteases, kinases, etc. On the other hand, it is tempting to speculate whether drug candidates can also be found in regions of the chemical universe which are, so far, not populated by drugs. Considering the failure of early combinatorial chemistry, driven by chemical accessibility instead of drug-like character of the products, it still seems to be more rewarding to search in areas that are already known to deliver drug candidates; it might well be that drug space is not evenly distributed within chemical space. □

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