Navigating chemical space for biology and medicine

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Despite over a century of applying organic synthesis to the search for drugs, we are still far from even a cursory examination of the vast number of possible small molecules that could be created. Indeed, a thorough examination of all 'chemical space' is practically impossible. Given this, what are the best strategies for identifying small molecules that modulate biological targets? And how might such strategies differ, depending on whether the primary goal is to understand biological systems or to develop potential drugs?

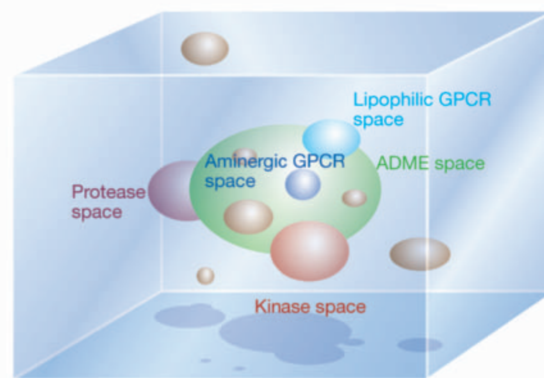
The relationship between chemistry, biology and medicine has been remarkably productive over the past century, since Paul Ehrlich pioneered the idea of systematically searching for drugs. By screening just over 600 synthetic compounds, Ehrlich discovered arsphenamine (Salvarsan)[1], which greatly improved the treatment of syphilis. Researchers now routinely screen millions of compounds in the search for some that are biologically active. Yet even the compound files of the largest pharmaceutical companies (which typically contain approximately 10^6 compounds) offer only a cursory examination of all the possible organic compounds that comprise 'chemical space' (Box 1). Chemical space is for all practical purposes infinite and limited only by the chemist's imagination.

Not all biologically active compounds have the desired physicochemical properties to be a drug. A biologically active compound may be too lipophilic (greasy) to be orally absorbed, too polar to cross the gastrointestinal wall or may have too much vulnerable chemical functionality that can be attacked by metabolizing systems in the liver, and therefore not remain intact for long enough to have a useful *in vivo* effect.

Box 1
Chemical space

Chemical space can be viewed as being analogous to the cosmological universe in its vastness, with chemical compounds populating space instead of stars. For example, there are more than 10^{29} possible derivatives of *n*-hexane — if we use a list of only 150 substituents and consider mono- to 14-substituted hexanes[50]. However, not all theoretically postulated compounds fall within the limits of what is synthetically feasible to produce, even with our current, extensive knowledge of organic chemistry. To navigate the vast diversity of chemical space, the concept of 'chemography', which is akin to a global positioning system, has been proposed. This involves mapping compounds onto coordinates of chemical descriptors of various physicochemical or topological properties[51,52]. Given the vastness of chemical space, the challenge for chemical biologists and drug discoverers is to identify those regions that are likely to contain biologically active compounds, that is, biologically relevant chemical space. The limits of biologically relevant chemical space are defined by the specific binding interactions between small molecules and the three-dimensional molecular recognition patterns on biological molecules, such as proteins, RNA and DNA, which have evolved over billions of years.

Measured in terms of physicochemical properties and topological descriptors, therapeutically useful compounds appear to cluster together in galaxies. A major unknown is whether these galaxies are evenly and sparsely distributed and therefore hard to find, or whether most of the chemical universe is 'empty' (containing no therapeutically interesting compounds), with galaxies of therapeutically interesting compounds scattered far apart. A century of medicinal chemistry and thousands of high-throughput screening programmes suggests that compounds that bind to certain 'target classes' (proteins from the same superfamily, such as G-protein-coupled receptors; GPCRs) are clustered together in discrete regions of chemical space (see figure). These regions can be defined by particular chemical descriptors.



Box 1 Figure The figure depicts a cartoon representation of the relationship between the continuum of chemical space (light blue) and the discrete areas of chemical space that are occupied by compounds with specific affinity for biological molecules. Examples of such molecules are those from major gene families (shown in brown, with specific gene families colour-coded as proteases (purple), lipophilic GPCRs (blue) and kinases (red)). The independent intersection of compounds with drug-like properties, that is those in a region of chemical space defined by the possession of absorption, distribution, metabolism and excretion properties consistent with orally administered drugs — ADME space — is shown in green (see Box 2).

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Box 2
What do drugs look like?

Drug-likeness
The distribution of the molecular properties of small-molecule launched drugs has changed little in the past 20 years, despite changes in the types of clinical indication for which drugs have been discovered and the range of targets acted upon[53]. Lipinski's seminal analysis of the Derwent World Drug Index introduced the concept of drug-likeness: orally administered drugs are far more likely to reside in areas of chemical space defined by a limited range of molecular properties. These properties have been encapsulated in Lipinski's 'rule of five'. This analysis shows that, historically, 90% of orally absorbed drugs had fewer than five hydrogen-bond donors, less than ten hydrogen-bond acceptors, molecular masses of less than 500 daltons and log P values (a measure of lipophilicity) of less than five[2]. Since this work, various definitions of, and methods to predict, drug-likeness have been proposed[2,54–65]. However, the consensus is that drug-likeness is defined by a range of molecular properties and descriptors that can discriminate between drugs and non-drugs for such characteristics as oral absorption, aqueous solubility and permeability. Computational property filters can be used to rapidly assess the drug-likeness of chemical libraries *in silico* before purchase or synthesis[21].

Druggability
The concept of druggability postulates that since the binding sites on biological molecules are complementary with their ligands in terms of volume, topology and physicochemical properties, then only certain binding sites on putative drug targets will be compatible with high-affinity binding to compounds with drug-like properties[31]. The extension of this concept to a whole genome analysis leads to the identification of the druggable genome. This is the expressed proteome predicted to be amenable to modulation by compounds with drug-like properties[30].

Recently, toxicity has replaced poor drug metabolism properties as a major cause of failure in the early clinical phase of drug discovery.

The determination of the characteristics of compounds that are more likely to yield safe, orally bioavailable medicines has led to the concept of 'drug-likeness'. Compounds that are drug-like have the potential to be developed into orally administered drugs (Box 2; ref. 2), which are generally favoured owing to their ease of use by patients. But biologically active compounds that do not have the exacting properties required of a drug can nevertheless be extremely useful to science as 'tools' for dissecting biological mechanisms and testing hypotheses in model systems. In recent years, it has been argued that it would be useful to discover a chemical tool to modulate every known protein[3]. Indeed, the Molecular Libraries Screening Center Network that is being established as part of the recent National Institutes of Health (NIH) Roadmap is aiming to facilitate the discovery of new chemical tools to understand biology, some of which may aid future drug development[4]. This Roadmap will allow the public sector to obtain data from high-throughput screens of a large

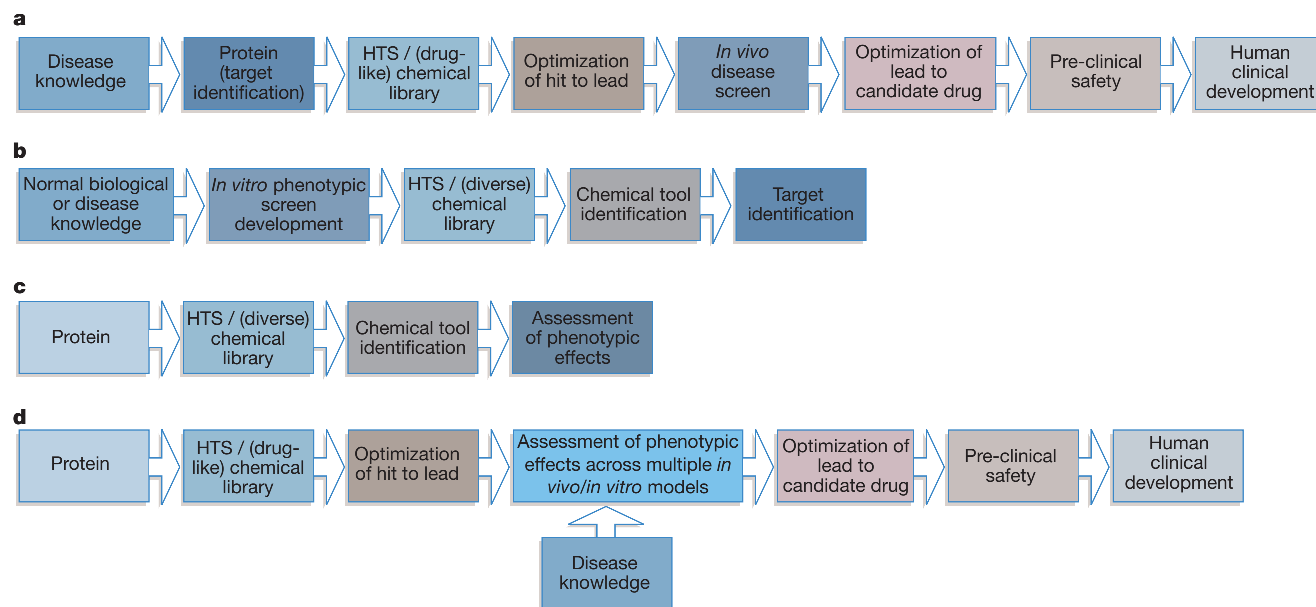

Figure 1 A comparison of approaches to discovering small-molecule tools or drugs. **a**, The 'standard model' of drug discovery is considered to be a linear process. New targets (usually proteins) are identified through knowledge of a particular disease. Compounds in drug-like (see Box 2) chemical libraries are tested in high-throughput screens (HTS) for their ability to bind to or modulate the target of interest. Selected initial hits (compounds that show levels of activity beyond a certain threshold level in the screen) are subsequently optimized through testing in further screens (often lower throughput) to give leads that have the required pharmacokinetic properties. These are then tested *in vivo*. Leads showing the required efficacy in *in vivo* disease models are further optimized into clinical drug candidates, which are then tested in human clinical trials. **b**, By comparison, forward chemical genetic approaches to developing chemical tools often start by screening a diverse chemical library to identify chemical tools that induce a particular phenotypic effect (such as cell death or cell proliferation). In phenotypic screens, the specific target of the chemical tools is often unknown, so a subsequent stage of target identification is required. **c**, Reverse chemical genetic approaches begin with a target of interest and then attempt to discover a specific chemical tool that binds to the target, usually by screening a diverse chemical library against the target *in vitro*. The specific chemical tool is then assessed for its ability to cause a range of phenotypic effects to identify the function of the target. **d**, Combining chemical tool and drug discovery approaches can result in an alternative drug discovery strategy to the standard model. Here, specific chemical tools are screened empirically across several disease models to discover new therapeutic effects.

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Table 1 **Rough guide to some major *in vivo* biological tools**

Biological Tool	Description	Advantages	Disadvantages	Reference
Protein-specific antibodies	Immunoglobulins secreted from a single clone of antibody-producing cells can be used as immunochemical reagents for the detection or assay of particular antigens	• Highly specific for intended target • Can be generated for virtually any species with known immunoglobulin genes	• Specifically target only circulating and cell-surface proteins *in vivo* • Lack of cross-species selectivity with some targets	34
Engineered recombinant proteins	A technique of subtly modifying endogenous proteins to produce ligands with agonist or antagonist functions	• Single amino-acid changes are often sufficient to produce antagonist ligands from naturally secreted proteins	• Predominantly target circulating and cell-surface proteins *in vivo* • Lack of cross-species selectivity with some targets	35
Gene knockouts	A technique in which homologous recombination is used to inactivate a gene from its endogenous locus to create a null phenotype	• Applicable to routine mass production of gene knockouts in mice	• Irreversible gene knockouts are not capable of mimicking the temporal and dynamic nature of perturbation that is characteristic of chemical or protein tools	36
Gene knockins	A technique in which homologous recombination is used to insert a gene into a selected chromosomal location	• Engineered site-directed mutant knockins can be used to design selective chemical-tool–receptor systems • Can be used to induce gain-of-function mutants to mimic agonist activity	• Initial chemical tool and/or structural knowledge of binding site required for site-directed engineering • Gain-of-function mutants are permanent, so do not mimic the dynamic nature of agonism	37
RNA interference	A phenomenon in which the expression of a gene is temporarily inhibited when a double-stranded complementary RNA is introduced into the organism	• Targeted libraries can be produced on a genome-wide scale for mammalian cell systems • Mass-production *in vivo* RNAi methods available for worm, fly and zebrafish	• Introduction of foreign double-stranded RNA into cells often results in an interferon response *in vivo* and other off-target effects • Genes with high messenger RNA turnover/low protein turnover can be difficult to target • Chronic *in vivo* administration difficult owing to delivery by large-volume hydrodynamic intravenous injections	38
Intrabodies	Intracellular antibodies composed of single-chain Fv fragments that consist of heavy- and light-chain antibody variable domains	• Target previously inaccessible intracellular proteins • Act at protein level, interacting directly with target of interest • Potential to act as agonists as well as antagonists	• Whole-animal *in vivo* delivery using viral vectors or liposomes is currently problematic	39

collection of compounds (initially about 500,000 compounds) in various biological assays. Here, we consider the scientific and practical issues that need to be addressed if efforts to discover new chemical tools are to provide the maximum possible benefit.

Chemical tools versus biological tools

Before the molecular biology revolution, the tools of the pharmacologist were usually the only ones available for probing the behaviour of biological systems. The pharmacologist's tools were mostly chemicals, derived from natural sources or from chemical synthesis. Perturbations of biological systems using such tools, some of which led to the development of drugs, taught us much about biology. For example, the natural product staurosporine — used as an early tool to probe the effects of tyrosine kinase inhibition — was important in the discovery of the anticancer drug imatinib (Gleevec), an inhibitor of the BCR–ABL tyrosine kinase.

However, the discovery of a new pharmacological tool was, and still is, a relatively rare and somewhat serendipitous event. At the core of efforts to discover small molecules of biological interest is typically some form of biological screen, in which a collection of compounds (known as a library) is assayed for a particular biological activity. In the early era of pharmacology, the compounds were often derived from natural sources, and the assays were for effects such as antibacterial activity or anti-inflammatory activity, usually using *in vivo* primary screens. More recently, with the molecular biology revolution, screening against isolated macromolecular targets has become widespread, and the compounds screened are often purely synthetic products from combinatorial chemistry (an approach for creating molecules *en masse*) as opposed to natural products[5]. Indeed, since the publication of the first paper to describe the synthesis of a single combinatorial library in 1992 (ref. 6), there has been a considerable increase in the numbers of combinatorial-chemistry compounds being developed for high-throughput screening experiments. For example, the sixth annual Comprehensive Survey of Combinatorial Library Synthesis records a total of 338 chemical libraries published in 2002 — a 25% increase from the previous year[7]. The success of combinatorial chemistry so far is hard to assess because of the 10- to 15-year time lag between initial chemical synthesis and drug launch. However, the general consensus is that many of the compounds made in the early years of combinatorial chemistry (from about 1992 to 1997) were severely flawed. Growing appreciation of the underlying reasons for this has considerably improved current combinatorial chemistry. The lesson to be learned here is that a radically new technology such as combinatorial chemistry may take well over a decade to mature and become fully useful.

Screening of small molecules is still the technology of choice for the development of many human medicines (Fig. 1), owing to its compatibility with the production of orally administered drugs. But for investigating biological function, biological tools have been in ascendance. These are created by genetic and protein engineering techniques, and are both cheaper and more efficient to develop than small-molecule chemical tools. Over the past decade, biologists wishing to probe protein function have invented an ever-growing array of techniques to manipulate and perturb biological systems (see Table 1).

It is estimated that the databases of the world pharmaceutical companies collectively contain small-molecule compounds known to directly modulate the function of only around 1,000 proteins (although few small-molecule compounds would be considered wholly selective)[8]. However, using genetic techniques, biologists can now readily selectively delete or silence the expression of almost any gene in the genomes of several diverse model organisms, including yeast, worm, fruitfly, zebrafish and mouse. Such genetic methods to explore the function of specific genes are on average 10 to 1,000 times

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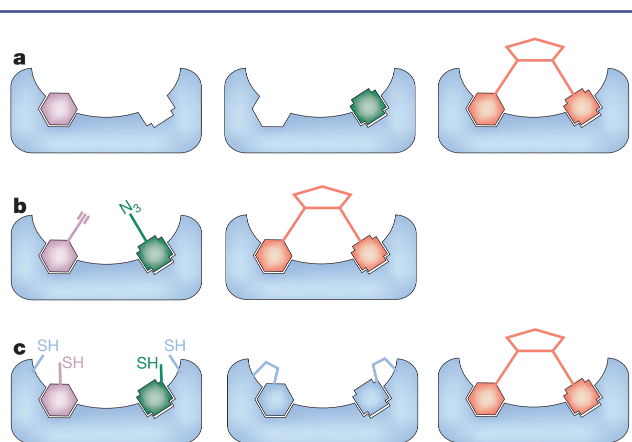

Figure 2 Fragment-based lead screening. Methods are currently being developed to more effectively search chemical space by screening a relatively small number of low-molecular-weight 'fragments'. Although in theory an unimaginably large number of drug-like compounds can be synthesized, chemical space grows as a function of the number of atoms in the compound (that is, its molecular weight): as the number of atoms increases, so does the number of possible permutations. So, restricting the molecular weight by screening only fragments and scaffolds allows a much larger area of possible chemical space (for a given number of atoms) to be explored. Low-molecular-weight, weak-binding fragments are then connected to form high-affinity, higher-molecular-weight ligands[40]. Several approaches based on this idea have been developed. **a**, Nuclear magnetic resonance (NMR)[41], mass spectrometry[42] and X-ray crystallography[43–45] are used to screen for low-affinity fragments (shown in mauve and green). Information on the structure–activity relationships (SAR) from these approaches can be used to rationally link fragments that bind in different parts of the target binding site to give larger, high-affinity ligands (shown in orange). **b**, The binding site can also be used to 'guide' the self-assembly of fragments (shown in mauve and green) containing chemical groups that can react to link the fragments to give high-affinity ligands (shown in orange) — an approach known as 'click chemistry'[46,47]. **c**, An approach known as 'tethering' can also be used to identify fragments (shown in mauve and green) that form covalent disulphide bonds to engineered cysteine residues within the binding site[48,49]; again, these fragments can be combined to produce larger, high-affinity ligands (shown in orange). Approaches such as tethering can also help to identify small molecules that bind to protein targets in cases where high-throughput screening approaches have been unsuccessful (for example, the so-called 'undruggable' targets).

less expensive than current chemical-based methods (R. W. Spencer, personal communication). This is exemplified by the fact that it is possible for a small biotechnology company to produce knockout mice for every member of the 'druggable genome' (Box 2) in only a few years[9]. Even with the combined screening resources of the top ten pharmaceutical companies, several years and several billion dollars would be required to produce the equivalent number of chemical tools from screening for the same set of targets. This is illustrated by the following rough calculation: it is not uncommon in industry to screen one million compounds per high-throughput screening campaign; so, if the total screening cost was as low as US$0.4 per compound (R. W. Spencer, personal communication), including the cost of the chemical synthesis, high-throughput-screening disposables, capital costs and human resources, screening just 25 targets with one million compounds would cost US$10 million, and screening the estimated 2,500 druggable targets in the human proteome would cost approximately US$1 billion.

Despite advances in the development of biological tools, many such tools have severe limitations, particularly when it comes to investigating the dynamic, reversible and temporal elements of protein function. In addition, although biological tools can antagonize the function of a protein by preventing or reducing its expression, or by blocking its ability to bind to other proteins, few biological tools allow the mimicking of 'agonist' behaviour by causing gain-of-function. This can be achieved more readily in certain gene families, such as G-protein-coupled receptors, by using small molecules. The realization of these limits has resulted in a revival of traditional small-molecule approaches to understanding biological function. Such approaches — now re-branded as 'chemical genetics' or 'chemical genomics' — are similar in character to the empirical investigational methods of pharmacology and physiology (see Fig. 1)[3,10].

Chemical tools are also important for target validation in drug discovery; that is, they can be used to verify whether a protein is a suitable target for drug development. One assumption underlying the chemical genetics approach is that the chemical tools are sufficiently selective in their modulating activity that an altered function can be assigned to a specific protein. However, compared with the exquisite selectivity of many biological techniques, selectivity cannot be commonly assumed for small molecules[11,12]. Moreover, although our knowledge of the desired properties of chemicals intended to become drugs is growing, very little is known about the chemical characteristics required of tools when the goal is something other than drug discovery.

Drug discovery versus knowledge discovery

The desired properties of chemical tools in the broadest sense depend in part on the goal of the experimenter: chemical geneticists aim to use small molecules to explore biological function; those involved in drug discovery and development aim to find small molecules that achieve a desired therapeutic effect in humans without causing unacceptable side effects. The importance of this difference in goals can be appreciated by briefly contrasting the general approach and priorities of the pharmaceutical industry with the approach and priorities of academic laboratories involved in chemical genetics.

The current primary strategy of the pharmaceutical industry for identifying biologically active molecules that might be starting points for potential drugs is the use of high-throughput screening. Here, libraries of about 10^5 to 10^6 small molecules with some drug-like characteristics are screened in high-throughput assays. These assays measure the ability of the small molecules to modulate a particular biological target, and vast amounts of data are generated. However, what is perhaps not widely appreciated by those outside industry is the generally poor quality of these data. For example, when an identical set of compounds is screened against the same biological target using three different assay formats, the concordance in the number of biologically active compounds or 'hits' obtained from each assay is just 35%. This is due in part to the inherent noise in the assays[13,14], although reproducibility within each individual assay is much more robust. Nevertheless, this low quality is acceptable to industry, as long as some active compounds are identified that have the potential to be optimized using more rigorous, lower-throughput assays. In other words, the high-throughput-screening process merely serves as a coarse 'filter' on the route to locating a potential drug; the limited number of positive hits are used to direct further experiments. The 'negative' information is too coarse to falsify hypotheses such as whether a particular type of chemical structure does not have a particular effect, but it can be exploited to identify borderline hits by computational pattern recognition[15] and probabilistic data mining[16]. Several factors contribute to the limitations of negative data, such as the fact that, with rare exceptions, compound concentrations are unknown in high-throughput screening because of well-documented compound solubility problems, both in dimethylsulphoxide (DMSO) stocks and upon dilution with aqueous buffer[17]. A compound may therefore appear inactive because it is truly inactive, or simply because its concentration was much lower than that assumed.

By contrast, in chemical genetics studies carried out in academic laboratories, collections of small molecules are typically screened in

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assays for their effects on processes such as cell death, cell migration and cell proliferation. A key aim of these studies is to identify correlations between different experiments that will aid in understanding the basis of the biological activities observed. Such experiments are discussed extensively in the review in this issue by Stockwell (page 846); but the key point to appreciate here is that data quality for such experiments is crucial. Given this, what approaches might be the most appropriate for identifying new chemical tools?

Searching for the right chemical tool

Suppose that the goal is to interrogate a biological system with a small molecule and that we restrict ourselves to using only robust, positive information that has survived a filtering process of experimental re-testing. Furthermore, let us agree that we want to generate useful information in a tool sense; that is, our aim is to learn something about biological function, whether or not it has any relevance to human therapeutics. What properties does the tool need to have? Must we restrict ourselves to using drug-like compounds? Two chemical extremes can be discerned: tools with properties consistent with their development into oral drugs; and tools with properties that could confound their development into oral drugs. Of course, many compounds will lie between these extremes. By discussing both these extremes here, we attempt to illustrate the considerations that could be important for initiatives aimed at developing chemical tools to explore biology and/or to act as a starting point for drug development.

Tools with drug-like properties

At one extreme, the chemical nature of the tool itself is drug-like, although the tool does not necessarily have all the attributes required of a drug (see Box 2). The main advantage here is that, should modulating the target of an identified tool compound be of therapeutic interest, this tool compound will be a suitable starting point for drug development. Another advantage is that limiting the search for tools to drug-like compounds means avoiding the potential pitfalls associated with compounds that contain chemical groups associated with toxic effects, or compounds that interact covalently with protein targets. The latter suffer from problems such as lack of specificity and unsuitability for optimization by medicinal chemistry techniques[18,19]. Although several well-known drugs, such as omeprazole and β-lactamase inhibitors, are known to act by means of irreversible mechanisms[20], medicinal chemists and toxicologists are becoming more wary of incorporating reactive groups within tools or drugs that can form covalent bonds to the target and/or other proteins. A disadvantage of drug-like libraries is that the breadth of commercially available chemistry space is decreased by the order of 50% to 80% (ref. 21). Another disadvantage is cost; frequently, drug-like compounds are more expensive to purchase than non-drug-like compounds.

Tools that are not like oral drugs

At the other extreme, the tool is not drug-like; chemical 'flaws' are present that mean the compound is unlikely to be used to treat human disease. For example, a moiety associated with toxicity can be present in a tool, provided that the unwanted toxicity does not present itself in the timescale of experiments using the tool, or if the tool is only intended for use in systems where toxicity is not an issue. An advantage of this type of tool is that the commercially available chemistry space is larger. Another very considerable advantage is that the interrogation of biology is unhindered by other drug discovery considerations, such as the need for the tool compound to be orally bioavailable.

Nevertheless, chemical genetics requires selective tools to interrogate and dissect biological processes. Lack of selectivity in a tool with 'relaxed' chemistry criteria (that is, chemical structural features known to be associated with increased probability of drug discovery failure) is a very real possibility; chemical features associated with failure in drug discovery tend to cause compounds to have 'promiscuous' effects in biological systems. A clear example of this would be the presence of a functional group that is likely to interact covalently with proteins (such as an epoxide or an aldehyde) in a simple, featureless, low-complexity compound (the complexity of a compound is related to the character and number of functional groups within the compound; see also ref. 22). This is because a low-complexity compound has a higher probability of weak binding to a target and a higher probability of binding to many targets[23] . When a low-complexity compound irreversibly binds to many targets (for example, several proteins) by means of covalent chemical bonds, the complexity of the biological effects elicited is very large. Thus, deciphering the effects of the compound as a tool is difficult. Whereas biological tools can be designed to be exquisitely selective for a particular gene or protein, it is harder to make the same selectivity prediction for any small-molecule chemical tool[12,13].

Another disadvantage of chemical tools that are not drug-like is the lack of clarity as to whether chemical features will defeat the utility of the tool. The available chemical space is likely to increase as chemistry criteria are relaxed. But if the aim is to use the chemical tool in *in vivo* animal models, which may have more relevance to both normal biology and disease than *in vitro* systems, then consideration of the drug-like properties of the tool, in terms of pharmacokinetics and the therapeutic index between efficacy and toxicity, is vital.

Relationship between tools and models

Whether the aim is to discover drugs or to gain knowledge of biological systems, the nature and properties of a chemical tool cannot be considered independently of the system it is to be tested in. Compounds that bind to isolated recombinant proteins are one thing; chemical tools that can perturb cell function another; and pharmacological agents that can be tolerated by a live organism and perturb its systems are yet another. If it were simple to ascertain the properties required to develop a lead discovered *in vitro* to one that is active *in vivo*, drug discovery would be as reliable as drug manufacturing. Indeed, examples abound of experimental drugs with the same primary effect in an isolated *in vitro* assay (such as antagonism of a particular protein) failing in clinical development because of inappropriate pharmacokinetics and/or toxicity. For example, the first histamine H2 receptor antagonist to be tested clinically was burimamide. Its pharmacokinetic properties were not compatible with oral administration, but tested parenterally (administered in a manner other than through the digestive tract), it was used to prove that inhibiting histamine H2 receptors effectively inhibited gastric-acid secretion. Metiamide, the second H2 receptor antagonist tested in humans, was orally active, but clinical trials were terminated because it caused fatal bone marrow toxicity. Cimetidine was the third H2 receptor antagonist to reach the clinic. This orally active compound was devoid of the toxicity found in metiamide and became the world's first billion-dollar blockbuster drug; its safety is attested to by its eventual over-the-counter availability worldwide. In general (whether we are considering either tools or potential drugs) because of the uncertainty of whether a compound has all the required properties to act effectively at a specific point in a whole organism, we cannot falsify a hypothesis about the biological function of a specific protein unless dosing effects, pharmacokinetics and selectivity are understood. All this requires significant investment and investigation.

If our goal is to discover chemical tools that bind to isolated recombinant proteins, then several emerging chemical technologies based on screening low-molecular-weight chemical 'fragments' may allow a more effective exploration of chemical space than the high-throughput screening of large chemical libraries (new approaches in this area are discussed in Fig. 2 and Box 3). However, the 'reductionist' approach of screening for small-molecule hits in isolated assay systems that bear little resemblance to the biological systems in which they are meant to act may be partly responsible for the decline in drug discovery productivity of the pharmaceutical industry over the past decade[24,25].

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Box 3
Protein dynamics and chemical space

The experience of the pharmaceutical industry in screening thousands of protein targets indicates that not all proteins are amenable to small-molecule modulation. Those that are not are called undruggable targets. A great deal of investment can therefore prove fruitless. Nevertheless, occasionally an unexpected allosteric binding site for a drug or chemical tool is discovered. Therefore, methods to discover ligands for unpredicted binding sites could improve the cost-effectiveness of searches for chemical tools. A particular challenge is to identify *a priori* which undruggable proteins are flexible enough to accommodate allosteric binding sites. A more effective method might be to combine several technologies. For example, the computational ability to scan protein structures *in silico* for flexible 'hot spots' — protein features that are likely to interact with small organic molecules[32,66] — could be coupled with protein binding site analysis[31] and substantial improvements in 'docking and scoring'. The latter involves the computational prediction of the binding of small-molecule ligands to the structure of a protein derived from an experimental X-ray or NMR structure.

The reductionist approach is a powerful aid to medicinal chemistry, in terms of understanding the potency and selectivity of small molecules for particular isolated protein targets. However, it has been criticized for its over-emphasis on potency and selectivity; pharmacokinetic properties, toxicity and biological responses have, in general, been beyond our ability to model or predict. Indeed, in drug discovery, despite the existence of stringent drug-like criteria, drugs and leads are rarely wholly selective for one target. In fact, 'poly-pharmacology' is often the basis for a drug's efficacy (as illustrated by many psychiatric compounds and a growing realization of the role of promiscuity in the generation of new kinase inhibitors in oncology[26]).

The most efficient strategy for finding chemical tools or drugs is often mistaken for the most effective strategy. Although the modern reductionist *in vitro* methods used in early discovery are efficient at discovering hits against new, isolated targets, it seems more difficult to convert such hits into drugs. This might also be a challenge for those trying to develop effective tools for probing *in vivo* biology. However, historically, the problem of the *in vivo* screening approach has been the difficulty in discovering new leads for new targets. The use of phenotypic *in vivo* screens as primary assays requires a compound to have suitable absorption, solubility and permeability characteristics, in addition to high potency at a given target and relatively low toxicity, for activity to be detected. Nevertheless, as our understanding of the properties of drugs, as distinct from the rest of the chemical universe, increases (see Box 2), lessons from the historical approach are being applied to high-throughput screening assays and chemical-library design for drug discovery[27]. Such knowledge could also be valuable in the development of chemical tools.

Perspective

Is the goal of discovering a small molecule to modulate every known protein practical? Decades of medicinal chemistry experience within the pharmaceutical industry suggest otherwise. Although less-stringent criteria applied to chemical tools can expand the accessible biological target space, toxicological and pharmacokinetic considerations must be taken into account if the use of a tool is to extend beyond isolated protein-binding assays to probing whole animal systems. So, for some targets, it may be more cost-effective to generate biological tools.

Some argue that the only limit to developing a chemical tool for a given protein target is the diversity of the chemicals screened against it. However, the evolution of specific molecular recognition by proteins creates stringent physicochemical limits that restrict the target set available to modulation by small molecules. These constraints are more severe if the aim is to discover drugs that can be orally administered. Furthermore, while many pharmaceutical companies and academics have been following the assumption that using a larger array of diverse chemistry to explore wider areas of chemical space will lead to the discovery of new drugs, the most successful drug discoverer to date, the late Paul Janssen, was surprisingly conservative in his exploration of chemical space[28]. Janssen's drugs show a steady evolution in their structures because he understood the constraints of biological activity, pharmacokinetics and toxicology on chemical space. Janssen's conservatism in chemical space provided an anchor from which he could be more creative in exploring indications (uses) of a drug through clinical experiments and observations. It is this multiplicity of constraints and competing demands on drug discovery that led another great drug hunter, Sir James Black, to advise: "the most fruitful basis for the discovery of a new drug is to start with an old drug" (refs 8, 29).

Given our limited resources, is the best strategy to explore as much of the diversity of chemical space as possible, or to focus our explorations? In our view, a concerted effort by industry and academia to develop chemical tools to modulate those proteins that make up the predicted druggable subset of the proteome[30], and to make these available to all researchers, along with relevant pharmacokinetic data (for *in vivo* models), is an achievable goal that would be of great benefit to biological and medical research. We believe that this goal should be prioritized before resources are expended in the search for chemical tools to modulate proteins that are inherently less tractable to this approach (Box 3). Examples of such proteins are the many proteins that participate in protein–protein interactions in biological signalling cascades[31,32]. Alternative approaches, such as the use of monoclonal antibodies, might well be more cost-effective for such targets.

The discovery of new pharmacological tools may depend on the serendipity of screening until more effective design methods are devised (Fig. 2). Ultimately, our explorations of biologically relevant chemical space are not limited by our chemical imagination, but by the limits of protein architecture and flexibility (Box 3)[33]. Improving our ability to discover new chemical tools and medicines will require combining the efficiency of exploration gained by reductionism with the effectiveness of approaches that study biological systems as a whole. □

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