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Perspective

Probing the Probes: Fitness Factors For Small Molecule Tools

Paul Workman[1,*] and Ian Collins[2,*]
[1]Signal Transduction and Molecular Pharmacology Team
[2]Medicinal Chemistry Team
Cancer Research UK Centre for Cancer Therapeutics, The Institute of Cancer Research, Haddow Laboratories, Sutton, Surrey, SM2 5NG, UK
*Correspondence: paul.workman@icr.ac.uk (P.W.), ian.collins@icr.ac.uk (I.C.)
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Chemical probes for interrogating biological processes are of considerable current interest. Cell permeable small molecule tools have a major role in facilitating the functional annotation of the human genome, understanding both physiological and pathological processes, and validating new molecular targets. To be valuable, chemical tools must satisfy necessary criteria and recent publications have suggested objective guidelines for what makes a useful chemical probe. Although recognizing that such guidelines may be valuable, we caution against overly restrictive rules that may stifle innovation in favor of a "fit-for-purpose" approach. Reviewing the literature and providing examples from the cancer field, we recommend a series of "fitness factors" to be considered when assessing chemical probes. We hope this will encourage innovative chemical biology research while minimizing the generation of poor quality and misleading biological data, thus increasing understanding of the particular biological area, to the benefit of basic research and drug discovery.

Introduction: Why Probe the Probes?

Understanding Genomes, Normal Biology, and Disease Pathology

The publication of the human genome sequence was a landmark in biological research (Lander et al., 2001; Venter et al., 2001; www.ornl.gov/sci/techresources/Human_Genome/home.shtml). It provides the basis for understanding the role of all genes in normal physiology and disease pathology, particularly when coupled to the sequencing of the genomes of cancer cells (Stratton et al., 2009) as well as infectious organisms (Berriman et al., 2009) and other species (www.ensembl.org/info/about/species.html). Chemical tools are playing an important role, alongside molecular biology and genetic techniques, in functional annotation of the human genome (http://nihroadmap.nih.gov/molecularlibraries/index.asp; Austin et al., 2004) and in expanding the druggable genome (Hopkins and Groom, 2002; Overington et al., 2006). An ambitious and inspiring early grand challenge that was set for the emerging fields of chemical genetics and chemical biology was to identify small molecule probes for the products of all human genes (Schreiber, 1998).

The discovery and exploitation of chemical probes has evolved from the more traditional ad hoc pharmacological approach—where advantage was taken of individual natural products and drugs to explore cellular processes—to the current higher throughput approaches that are now in widespread use in both academia and industry (Austin et al., 2004; Frearson and Collie, 2009) and that facilitate interrogation of chemical and biological space in a more systematic way (Schreiber, 2003; Dobson, 2004). Although terms such as molecular pharmacology, chemical genetics, and chemical biology can be useful to indicate nuances of approach, the overall unifying principle is the use of small molecules to understand the function of genes and proteins and their role in physiology and pathology.

Synergies between Chemical Biology and Drug Discovery: Good Probes Are Key

There are major synergies between more basic chemical biology research and drug discovery (Anonymous, 2009). From a drug discovery perspective, chemical probes are key players in validating new molecular targets for therapeutic exploitation and in providing proof of concept for potential druggability of a molecular target, pathway, or process by small molecules. They can help to minimize the technical and biological risk for a biological target or pathway of interest. Chemical tools can also serve as pathfinder molecules in drug discovery projects, informing the design and evaluation of biological assay cascades and the identification of useful biomarkers.

Importantly, chemical probes are highly complementary to the use of RNA interference (RNAi), in particular in being able to inhibit a specific function of the target protein rather than removing the whole protein, thus avoiding multiple function or scaffold effect issues; in giving an immediate inhibition rather than a delayed knockdown; and in providing greater control over the extent and kinetics of inhibition (Weiss et al., 2007). Both chemical probes and the use of RNAi can have off-target as well as on-target effects. Use of these approaches in parallel, as well as making mutated alleles of target proteins (Bishop et al., 2000), can give us much greater confidence in functional annotation and target validation.

Over a long and distinguished history, the use of small molecule chemical tools has led to advances in biological understanding and therapy in such diverse areas as the cytoskeleton (colchicine, palitaxel), mitosis (monastrol), immunophilins and immunosuppression (FK506, cyclosporin), mTOR (rapamycin), histone deacetylases (trapoxin, vorinostat), protein kinases (phorbol esters, staurosporine, tyrphostins, and many others), PPARγ (thiazolidinediones, 2-chloro-5-nitrobenzanilide GW9662), and very recently, stem cell reprogramming (Emre et al., 2007).

Do We Need Rules and Guidelines?

From the examples cited above and others, it seems that the use of chemical probes has been extremely effective and is having a growing impact as a result of powerful new technologies. Chemical probes are clearly helping basic research and drug discovery. So why do we need rules or guidelines introduced into what seems to be a productive process? If it ain't broke why try to fix it?

Few would dispute that to be valuable in chemical biology and drug discovery research, chemical tools must satisfy at least some basic criteria, such as permeability (getting to the site of action in the cell), potency (inhibiting the target at reasonable concentrations) and selectivity (not being unacceptably promiscuous). The principle of "garbage in, garbage out" applies here big time. If a biologist uses a lousy probe, then the interpretation of biological results will likely be flawed.

Recent publications have suggested objective guidelines for what makes a useful chemical probe for application in biological research (Oprea et al., 2007; Cohen, 2009; Edwards et al., 2009; Frye, 2010; Kodadek, 2010). The emergence of guidance for probes is analogous to guidelines (sometimes referred to as rules, although they are more "rules of thumb") that have proved to be of real practical value for assessing the suitability of fragment or high-throughput screening (HTS) pharmaceutical leads for progression to drug candidates, and also for judging the candidates themselves (Lipinski et al., 2001; Oprea et al., 2001; Rees et al., 2004; Davis et al., 2005; Collins and Workman, 2006).

The potential need for guidelines for probes has been stimulated recently by the increase in public screening efforts and in particular, the assessment of the output of the large National Institute of Health Molecular Library and Imaging Initiative (NIH MLI) (http://nihroadmap.nih.gov/molecularlibraries/index.asp; Austin et al., 2004). In a high profile expert "crowdsourcing" review (Oprea et al., 2009) of the burgeoning output of the pilot phase of the NIH MLI that ran from 2004 to 2008 at an estimated cost of US $385 million, a significant proportion (25%) of the 64 chemical probes generated were considered to have a high "dubiosity" rating, with low confidence in their value as probes, whereas 25% and 50% were viewed as inspiring medium- and high-confidence, respectively. A recent conference presentation (Bologna, 2010) identified concerns with a high proportion of the NIH MLI hits and noted the very low citation rate for most NIH MLI probes proposed to date. Criteria for the nomination of a chemical probe were made more stringent during the pilot phase. Especially given the increasing involvement in the production and use of probes of scientists who may have less experience in this area than pharmaceutical industry professionals, the development of guidance for assessing potential probes does seem appropriate (see Kaiser [2008]).

On the other hand, nobody–and not just the more anarchic or rebellious fringe–wants a chemical biology thought police that dictates overly prescriptive rules that stifle innovation (Hoffmann and Bishop, 2010). Rules are unlikely to work. Moreover, as we will discuss later, probes evolve with time and need to be given a chance to be improved, especially in new research areas. A balance needs to be struck between allowing freedom for creativity and establishing sensible guidelines that eliminate at least the worst offenders among flawed probes and that encourage good practice in the community.

Finding the Balance and Examples from Oncology

Against the above background, we discuss the recently emerging views concerning the desirable properties of chemical probes. After reviewing proposed guidelines and offering our own recommendations, we will illustrate these with several case histories of biological targets that have benefited particularly well from the use of progressively enhanced chemical probes. The selection of these cases is made from the cancer therapeutic area because, with outcomes from the human genome sequence now being reviewed 10 years on from the initial announcement, cancer is seen clearly to be the area that has benefited most in terms of the discovery and implementation of personalized, genome-based medicines (Collins, 2010; Golub, 2010). Chemical probes have contributed considerably to the progress made with targeted cancer therapies and many of the drugs or analogs thereof have, in turn, served as small molecule tools for use in the lab. Note, however, that although the illustrative examples are taken from oncology, the views expressed on chemical probes should be of generic relevance across basic, translational and drug discovery research.

We advocate a "fit-for-purpose" approach to the properties of chemical probes, recommending "fitness factors" for probe evaluation. When combined with rigorous ongoing characterization and transparent reporting of the advantages and limitations of chemical probes together with open availability of reagents and data, a flexible, evidence-based strategy that is geared to the current understanding of the particular biological area should encourage innovative chemical biology research while minimizing the generation of poor quality and misleading biological data. Progressive characterization and iterative refinement of chemical tools by the international scientific community can then follow in parallel with increased comprehension of the particular biological area, to the benefit of basic and translational research and drug discovery.

Emerging Guidelines for Probes

In this section, we discuss recent proposals for the preferred properties of tool compounds and recommend what we term "fitness factors" for fit-for-purpose chemical probes. We build on previous guidelines that have been put forward for determining the use of chemical probes and the confidence in results derived from them (e.g., see Cohen, 2009; Frye, 2010; Kodadek, 2010 and references in the legend to Figure 1). Cohen and colleagues have particularly focused on choosing high quality protein kinase inhibitors for interrogating targets in cells, where selectivity of the agents is paramount (Cohen, 2009; Davies et al., 2000; Bain et al., 2003; Bain et al., 2007). Recognizing the challenge of specificity given the more than 500 protein kinases in the human genome, in his recommendations, entitled "guidelines", Cohen (2009) describes essential and desirable "criteria" for kinase probes. Frye (2010) was careful to draw a distinction between rules, which he argued were unrealistic in view of the numbers of parameters involved, in contrast to the more practical use of a small number of relatively simple "principles" or guidelines that could be of great value. These principles covered the biological and physicochemical properties needed in chemical probes that are intended for use in establishing the broader biological consequences of modulating a molecular target.

There is an overlap with established practice to weed out promiscuous compounds identified from HTS assays (Baell and Holloway, 2010; Inglese et al., 2007), especially in avoiding unspecific chemical reactivity (Rishton, 2003) and aggregation due to poor aqueous solubility (McGovern et al., 2003). Avoiding these undesirable properties will reduce the risk of a chemical probe exhibiting nonspecific effects. The importance of emphasizing these issues, which are well known in the pharmaceutical industry, is illustrated by the fact that significant problems with these properties were identified in the recent assessment of NIH MLI probes (Oprea et al., 2009; Bologna, 2010).

Specific guidelines for probes from the NIH MLI program are available (Lazo et al., 2007; Oprea et al., 2007; http://mli.nih.gov/mli/wp-content/uploads/probe-guidelines-v2-accepted-by-sc-200707.doc). It is interesting that in the recent crowdsourcing analysis of NIH MLI probes there was a fascinating divergence of views on individual putative probes among the various expert panel members (see Supplementary Material in Oprea et al., 2009). Thus there will be an important element of judgment based on experience involved in the assessment of probes, even with guidelines available.

Fitness Factors for Chemical Probes

The various existing guidelines mentioned above are interpreted and augmented with our own suggestions for fitness factors in Figure 1. We define these fitness factors as the key properties of chemical probes that should be evaluated in relation to their intended use to give confidence that they are fit-for-purpose. The performance of a probe with respect to the separate fitness factors taken together provides an assessment of the suitability of a given probe and of potential uncertainties in interpreting biological data obtained with it.

Figure 1 highlights the four main categories into which the fitness factors are classified. Collectively these define the suitability of a given chemical probe for exploratory biology. The four main categories are: (1) chemical properties, (2) biological potency, (3) biological selectivity, and (4) context of use. The fitness factors within each class are discussed in detail below. We also raise questions of experimental use that are important to consider in assessing the quality of chemical probes. Whereas the fitness factors are expressed for convenience as a ''checklist'' and commonly discussed threshold values are presented in Table 1, we do not imply that a rigid adherence to fixed values is always appropriate. Rather, they are criteria that may be used to facilitate judgments of the robustness of data generated with

A

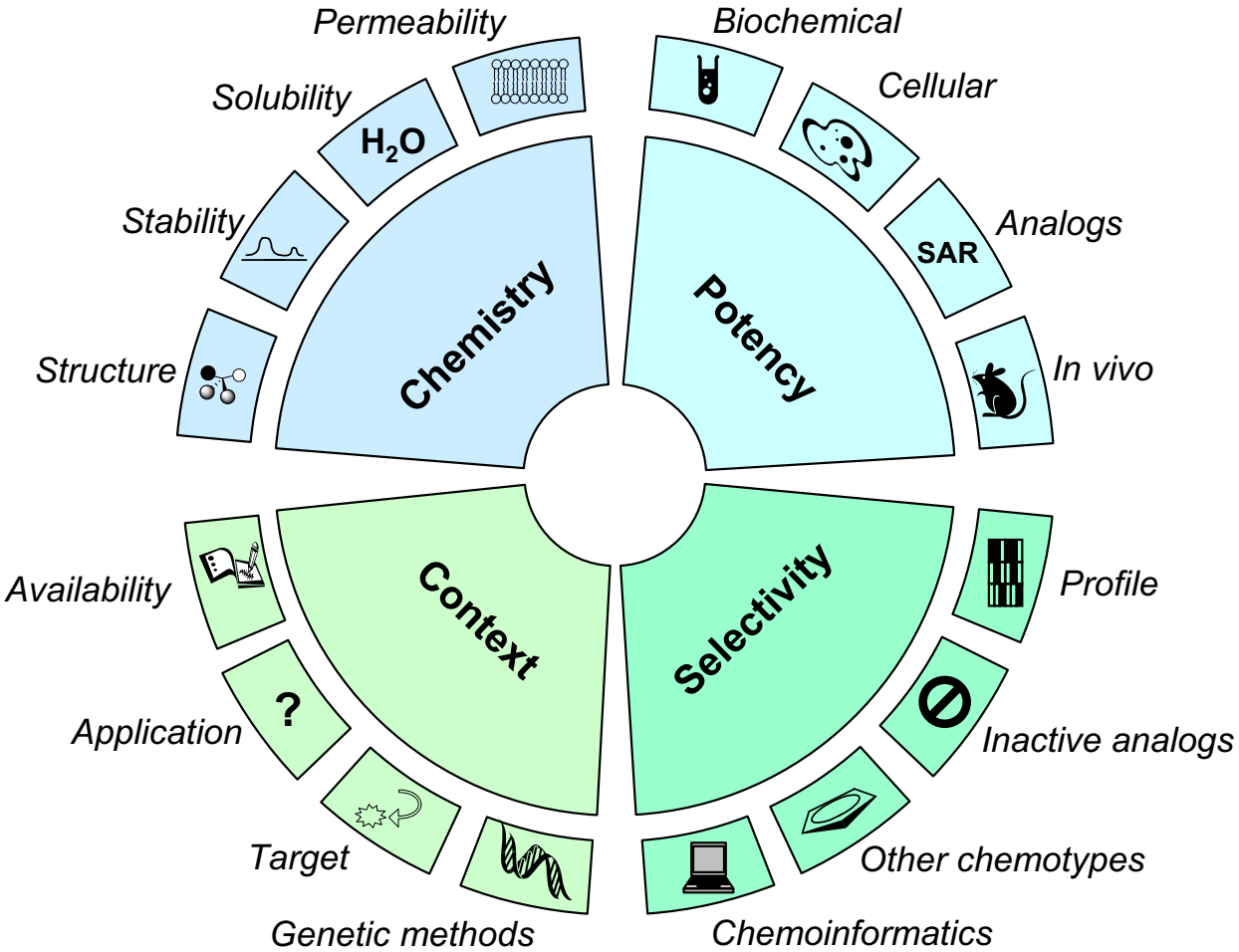


Figure 1. (*continued*).

B

	Chemistry	
	**Structure**	Discrete chemical species, characterised spectroscopically; Defined structure with reproducible preparative method
	**Stability**	Defined purity and stability in test media; Free from non-specific chemical reactivity
H_2O	**Solubility**	Sufficiently soluble in aqueous media; e.g. >100 μM; No aggregation effects in biochemical assays
	**Permeability**	Proven passive membrane permeability, or defined active transport mechanisms
	Potency	
	**Biochemical**	Typically <100 nM in an *in vitro* biochemical assay; Sufficient to confidently associate with cellular activity
	**Cellular**	Typically <1-10 μM in a mechanistic cell-based assay; Sufficient to confidently address hypotheses in cells; Concentration-dependent effect on the biological target
SAR	**Analogs**	Closely related structures identified with similar activity; Correlation of biochemical target activity and activity in cells; Correlation of biomarkers of target modulation with biochemical and cellular potency
	**In vivo**	Pharmacokinetic properties sufficient to achieve levels in target tissue relevant to cellular potency
	Selectivity	
	**Profile**	Defined selectivity for related targets or known targets of chemotype; Typically >10-100 fold in biochemical assay; typically >50 kinases profiled for kinase inhibitors; broader pharmacology profiling desirable
	**Inactive analog**	Analog with no biochemical target activity shows no activity in cells
	**Other chemotypes**	Probes from a different chemical class with similar activity
	**Chemoinformatics**	Awareness of other activities associated with the chemical class
	Context	
	**Genetic methods**	RNAi and/or mutants of target available for complimentary experiments
	**Target**	Cellular context of the target and potential linked activities considered
?	**Application**	Fitness of the probe to test the specific biological hypothesis considered
	**Availability**	Origin, identity and properties fully disclosed; Available for use without restrictions; Accessible in quantities (15-20 mg) for follow-up studies

Figure 1. Fitness Factors for Chemical Probes Grouped into Four Distinct Areas

Chemical properties, biological potency, biological selectivity, and context of use (A), that encompass suggested criteria for evaluating the suitability of chemical probe compounds for exploratory biology (see Baell and Holloway, 2010; Cohen, 2009; Edwards et al., 2009; Frye, 2010; Inglese et al., 2007; Kodadek, 2010; McGovern et al., 2003; Oprea et al., 2007; Rishton, 2003). Threshold values that have been suggested in the literature are tabulated for each of the criteria (B), and a comparison of these properties for drugs, leads, and probes can be found in Table 1. We suggest that although not all probes can, or need to, reach these thresholds in every case, consideration of the criteria will allow a robust assessment of whether the probe is fit-for-purpose, and foster an appreciation of the risk carried forward if significant criteria are not met.

particular probes in particular settings, and are intended to help estimate the risks that may be carried forward in building on data generated from the use of new chemical probes where, of necessity, incomplete characterization is available. Importantly, expertise in both chemistry and biology must be applied in this process.

The Big Four

Chemistry. The need for well-characterized chemical identity and purity of chemical probes is clear cut. It is also important that reliable and reproducible procedures for the synthesis of the compounds are available. The chemical stability of the compounds in relevant media should be evaluated, particularly

Table 1. Calculated and Experimental Properties of Drugs, Leads, and Probes

	Drugs[A,Co,G,O,L1,L2,W,Wo]	Leads[Co,K,L1,L2,O,Wo]	Probes[C,E,F, Ko,O]
Aqueous solubility	>10–100 μg/ml	>10–100 μg/ml	>0.05 μg/ml in low % DMSO aqueous solutions
Membrane permeability	10× permeability of mannitol in CaCo-2 assay desirable; minimal PGP-mediated efflux	$P_e > 10^{-6}$ ms^{-1} (in vitro assay); minimal PGP-mediated efflux	Permeability essential; minimal PGP-mediated efflux in cell lines of interest
Chemically reactive groups	None present unless a well characterized and selective mechanistic requirement	None present unless a well characterized and selective mechanistic requirement	None present unless a well characterized and selective mechanistic requirement
Molecular weight (Da)	<500–550	<350–450	Likely to be <450
Lipophilicity (LogP)	<5	<4	Likely to be <5
H-bond donors (O-H, N-H)	= <5	<4–5	Likely to be <3
H-bond acceptors (N, O)	= <10	<8–9	Likely to be <11
Rotatable bonds	= <10	<8	Likely to be <10
Target potency (IC_{50} or Ki)	10^{-8}–10^{-9} M	10^{-6}–10^{-8} M	10^{-7}–10^{-9} M
Ligand efficiency	NA	>0.3 kcal mol^{-1} heavy atom^{-1}	NA
Target selectivity	Well-defined selectivity; polypharmacology acceptable	Well-defined selectivity; >10-fold over related targets; minimal activity on common off-targets, e.g., HERG	Well-defined selectivity; >10–100-fold against closely related targets; polypharmacology undesirable
Mechanism of action	Activity in a relevant model of the target disease	Well-defined quantitative relationship between biochemical and cellular effects consistent with target-dependent action	Well-defined quantitative relationship between biochemical and cellular effects consistent with target-dependent action
Pharmacokinetics	Well-defined therapeutic window and in vivo pharmacokinetics	Stable in microsomes; no CYP450 inhibition	Good pharmacokinetics not essential for in vitro and cellular use, but required for in vivo animal work

For references to specific suggested criteria see: A, Amidon et al., 1995; C, Cohen, 2009; Co, Collins and Workman, 2006; E, Edwards et al., 2009; F, Frye, 2010; K, Kerns and Di, 2008; Ko, Kodadek, 2010; L1, Lipinski et al., 2001; L2, Lipinski, 2003; O, Oprea et al., 2007; W, van der Waterbeemd, 2002; Wo, Wohnsland and Faller, 2001.

CaCo2: human epithelial colorectal adenocarcinoma cell line, CaCo-2: CYP450: cytochrome P450 enzymes, DMSO: dimethylsulfoxide, HERG: human ether-a-go-go related gene product (K_v11.1 potassium ion channel), IC_{50}: half maximal inhibitory concentration, Ki: inhibitor dissociation constant, LogP: octanol-water partition coefficient, NA: not applicable, P_e: apparent permeability, PGP: p-glycoprotein.

with regard to sensitivity to acid or base. Covalently reactive functionality may be less problematic for chemical probes than drugs (Kodadek, 2010), especially if linked to a specific target mechanism, but caution is needed because nonspecific chemical reactivity may promote stress-related phenotypes in the cell. Thus a lack of chemical functional groups and physicochemical properties known to elicit oxidative stress, redox chemistry, membrane destabilization, or irreversible protein binding is very desirable (Price et al., 2009).

Aqueous solubility and membrane permeability are of great importance in providing usable chemical matter for cell-, tissue-, or whole organism-based research, and assays for these properties are now readily accessible (Kerns and Di, 2008). For leads and drugs, adequate solubility and permeability are required to achieve intestinal absorption and oral bioavailability but they are also essential for probes of intracellular targets to be useful in vitro. The achievement of pharmaceutical lead-like pharmacokinetic properties may not be absolutely essential in a chemical probe, but there are considerable advantages in probes that can be progressed with confidence to an in vivo whole animal setting. Similarly, the avoidance of adverse off-target toxicological effects is important in a tool for use in animal models; physicochemical properties as well as toxicophore groups are important for this, with an increased likelihood of toxic events being observed for less polar, more lipophilic compounds across a broad range of chemical space (Hughes et al., 2008). Thus the stringency of the fitness factors for in vivo probes is higher than for biochemical or cellular probes.

The value of defining the physicochemical behavior of compounds in drug discovery is well established. The widely used Lipinski guidelines (known as the "rule of five") are aimed to increase the likelihood that small molecules will have physicochemical properties compatible with the biological environment, especially for oral bioavailability (Lipinski et al., 2001). On the other hand, such probabilistic guidelines have many exceptions and must be applied in context, and similar diligence should be applied when assessing chemical probes according to physicochemical parameters. Evidence-based guidelines for the desirable properties of pharmaceutical lead-like molecules are summarized in Table 1 and compared with those for typical drug-like molecules (Collins and Workman, 2006) and those emerging for chemical probes.

The discrimination between lead-like (or fragment-like) and drug-like chemical space in terms of calculated molecular properties (size, polarity, conformational rigidity, efficiency of biological interaction) has been useful in drug discovery where

a fragment or HTS lead is anticipated to grow in size and complexity as it is engineered to a clinical candidate. A satisfactory probe may arise as a primary HTS hit and not require addition of further functionality to enhance properties, although iterative improvement to refine the probes is increasingly expected (Edwards et al., 2009). Analyses of the footprint of existing probes in chemical property space has shown interesting concordance with that of high quality pharmaceutical leads (Oprea et al., 2007; Lipinski et al., 2001) and there is recognition that an iterative process of refinement of initial HTS hits is required to generate high quality chemical probes—just as for the progression of a pharmaceutical lead to a drug.

Although the experimental performance of a molecule is the prime consideration, calculated property values may be useful in guiding the design of new screening libraries for both pharmaceutical leads and chemical probes to increase the probability of finding fit-for-purpose compounds. However, the necessarily retrospective nature of such analyses biases the definition of chemical space toward what is well known. Because chemical probes are intended to be used at the leading edge of biological research, it will be important to revise definitions of appropriate chemical space to encompass as yet relatively unexplored classes of target interactions, e.g., protein–protein interaction inhibitors (Dobson, 2004; Sperandio et al., 2010).

Potency. The biological potency of a probe should be appropriate to the intended research. A useful consensus has emerged in favor of <100 nM potency against the usually recombinant or purified biochemical target and at least 1–10 μM potency in the relevant cell-based system (Table 1) (Oprea et al., 2007; Frye, 2010; Edwards et al., 2009). A concentration-dependent effect of the probe on the target gives confidence in a targeted mechanism (Frye, 2010), and this may be supplemented by the availability of close analogs of the probe with varying degrees of activity. If a probe is to be useful for in vivo experiments in whole animals then the relationship of the potency to the in vivo pharmacokinetic properties should be evaluated to provide confidence that sufficient levels of the compound will be achieved in the target tissues. The availability of a suitable pharmacodynamic biomarker that shows engagement of the chemical probe with the target in cells and also in vivo in animal models is very valuable in this context (Workman, 2003).

Selectivity. The selectivity of probes is very important because they are intended as highly specific pharmacological modulators (Cohen, 2009; Frye 2010). It could be advisable to reconsider the selectivity profile of existing probes each time they are used in new biological settings where distinct confounding factors may be present (see more on context below).

The typical selectivity criteria for refined chemical probes (>10–100-fold over related targets; Table 1) may be more stringent than those for pharmaceutical leads because no further engineering of the former molecules is envisaged, and therapeutic polypharmacology is more likely to be acceptable (and sometimes may even be essential, as in multi-targeted protein kinase inhibitors) in a drug (Kodadek, 2010).

Large scale in vitro selectivity profiling is often recommended, particularly for kinase inhibitors (where several tens or hundreds of kinases may be tested) but also for modulators of other protein superfamilies (Fabian et al., 2005). We suggest that selectivity testing against at least 50 carefully chosen kinases is appropriate for assessing kinase inhibitor probes. Although this profiling can yield valuable information, it is important not to generate a false sense of security. For example, biochemical kinase inhibition profiles require interpretation to predict the likely cellular consequences (Smyth and Collins, 2009) and inhibitors may interact with other target families that may not be looked at (Bantscheff et al., 2007). Nevertheless, at some point on their journey chemical tool compounds ideally need to be well characterized in terms of broad ligand pharmacology, including effects on G protein coupled receptors, nuclear receptors, ion channels, kinases, phosphatases, proteases, and ubiquitin ligases (Entzeroth et al., 2000). Such broad profiling can rule out promiscuous pharmacology or activity on particular anti-targets.

The cost of selectivity screening in focused or broader pharmacology panels may be restrictive, particularly for academic groups and small companies. Useful pharmacological data on approved drugs can often be found in the disclosures that accompany regulatory approval (www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm). Nonbiased global gene expression profiling can provide a valuable means of assessing on- and off-target effects in cells, with connectivity maps linking the profiles to targets (Clarke et al., 2001; Lamb et al., 2006; http://www.broadinstitute.org/genome_bio/connectivitymap.html).

Selectivity screening can be supplemented by using control compounds in biological experiments, especially inactive or weakly active analogs from the same chemical scaffold, sometimes conveniently referred to as a chemotype, together with active analogs of a different chemotype. Examining concentration-dependency and structure-activity relationships (SAR) from these analogs compensates for the deficiencies of any one compound and thus triangulates the target-dependent phenotype, especially when the SAR for molecular biomarker changes that are associated with target modulation also correlate with cellular outcome (Cohen, 2009; Frye, 2010; Kodadek, 2010). This can be seen as an application of the "pharmacological audit trail"—used to build confidence during drug development—at the biochemical and cellular level (Workman, 2003; Yap et al., 2010).

These days, chemical probes frequently arise from large scale library screening that will often produce clusters of chemically related compounds of varying activity, so access to suitable analogs is not necessarily restrictive for academic groups (Inglese et al., 2007). Interrogating the burgeoning databases that annotate chemical structure with biological information will also inform on potential selectivity issues (Tolliday et al., 2006; Huryn and Cosford, 2007; Petri Seiler et al., 2008; Keiser et al., 2009; http://www.ebi.ac.uk/chembl). It will be important to apply the fitness factors under discussion here in evaluating historical data retrieved by such chemoinformatics methods, and to assess the reliability of the associations uncovered depending on the suitability and application of the probes concerned.

Context. Biological context is everything when discussing chemical tools. In stressing this, we emphasize that the appropriateness of a probe's use cannot necessarily be extrapolated from one biological system to another. The intended use will govern the suitability of a probe as well as the compound's intrinsic properties (Table 1). What is desirable is a suite of chemical probes and techniques, appropriate to the target and scientific question under investigation, i.e., fit-for-purpose, that allows

a consensus to be built about the role of a given target in a particular mechanism from multiple approaches. Thus complimentary genetic methods, such as specific RNAi or mechanism-based mutants of the target protein are recommended to reinforce the data from chemical probes while remaining aware of the differences between the techniques (Weiss et al., 2007; Cohen, 2009; Frye 2010).

An example of the dependence on context in oncology is the assessment of the activity of targeted probes or drugs in large panels of cancer cell lines with defined mutational, gene expression and other molecular characteristics (see http://www.sanger.ac.uk/genetics/CGP/cosmic/ for information on cancer gene mutations). Markedly differential effects on cell proliferation or apoptosis are seen depending on a given compound's selectivity profile combined with the cell's genetic/molecular profile (Sharma et al., 2010). Indeed, there is considerable power in interrogating many different cell types with a range of well-validated probes. The chemical probes are used to help understand the biology of the cell lines and the effects in the cell lines help to credential the probes (http://www.sanger.ac.uk/genetics/CGP/Translation).

That repetition of biological profiling in multiple relevant biological systems and in different laboratories is a key part of chemical probe validation is to be emphasized. An important fitness factor for context is to consider the question: is this the best probe to use for this purpose? There may be several probes available targeted to a particular pathway or molecular target, and the properties of certain compounds may be better matched to some biological systems than others and more capable of yielding a robust test of the biological hypothesis under investigation. For example, minimizing the risk of inhibition of particular confounding anti-targets in the biological system by avoiding a chemotype with those activities.

In addition to the uses of biomarkers described earlier, considerable value can be added to the probing of probes by using biochemical and molecular biomarkers to characterize the target cells of interest and then to evaluate the effects of the probes for on-target and off-target pathways in those cells (e.g., Banerji et al., 2005; Guillard et al., 2009; Raynaud et al., 2009). Biomarkers provide an important step in the pharmacological audit trail referred to earlier, helping to establish that the chosen chemical probe is fit for purpose in a specific context. Ideally, quantitative biomarker methods should be used so that the degree of target modulation can be determined and results interpreted accordingly. The interpretation of the effects of a probe on a cellular phenotype (e.g., proliferation, apoptosis, differentiation, migration, or other specific functions) is much more powerful if validated biomarkers are used to demonstrate that the probe is actually modulating the intended target and cognate biochemical pathway (e.g., Solit et al., 2006). In addition, of course, the probe compounds can be used to validate the proposed biomarkers.

Probe Availability, Transparency of Reporting, and Funding

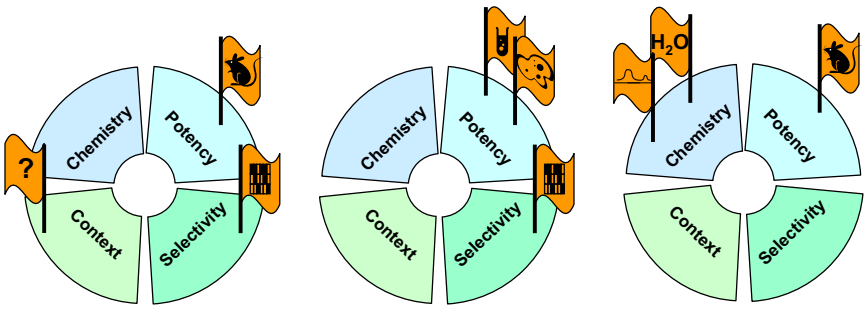
The origin, identity, and properties of a compound proposed as a chemical probe should be disclosed fully, and it should be available in appropriate amounts for follow-up studies. The different availabilities of chemical probes may in practice be the dominant factors in determining which compound is used, and availability is therefore an important fitness factor. Widespread availability of a probe and its associated data throughout the global scientific community is highly desirable. Provided that structures and synthetic methods are fully disclosed in scientific publications and patents, it should generally be possible to make the required compound, or have it made. There is an increasing tendency for probe compounds and drugs to be sold by commercial suppliers. Commercial supply can reduce the otherwise onerous demands on academic labs and companies to provide compounds from finite resources. Some pharmaceutical companies have taken the initiative of making probes available via commercial vendors (e.g., http://investor.sigmaaldrich.com/releasedetail.cfm?releaseid=451709). There may, however, be a need for new thinking to further improve the availability of probes throughout the research community, taking into account legal considerations around research use and supply of proprietary compounds. Obtaining larger quantities for animal studies can be especially challenging.

For a given target, we strongly recommend the use of probes from more than one chemical scaffold and also inclusion of negative control analogs. Despite their importance, companion inactive or other analogs are rarely available commercially. In the light of the selectivity fitness factors discussed above it could be desirable to consider chemical probes as reagent sets consisting of a pair of active and inactive compounds from the same chemotype, ideally together with an active compound from another chemical scaffold, in the same way that gene silencing studies using small interfering RNA reagents is typically conducted with multiple effective RNA oligomers together with appropriate negative control sequences.

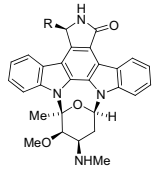
Several previous publications rightly stress the need for transparency in reporting all relevant data on proposed new probes as well as the desirability of unrestricted or ''open access'' availability (Oprea et al., 2007; Edwards et al., 2009; Cohen, 2009; Anonymous, 2010). Transparency on reporting findings is important so that data from all probes, whether the compound is freely available or proprietary, can be assessed with confidence and equal rigor.

What about funding for all this probing of probes? Very detailed profiling is expensive and the scale is increasing. Much of this work will be carried out using conventional support in academia and industry. Making probes widely available will mean that the international scientific community can put them through an enormous range of biological assays, thus building up a profile of information in an open access fashion that is similar to other fields such as software development and genome sequencing. Maintaining quality and having databases and tools to access this information is increasingly important (e.g., http://pubchem.ncbi.nlm.nih.gov/; www.ebi.ac.uk/chembl/).

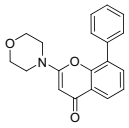
Special initiatives are also appropriate. The NIH MLI is one approach funded publically that seeks to build on success with large scale genome sequencing projects (Austin, 2003; Oprea et al., 2009). Oprea et al. (2009) discuss United States funding mechanisms that might be used to support probing the probes. Public–private partnerships have been initiated as a new funding model with the aim of developing open access chemical probes against pioneer targets such as chromatin modifying enzymes (Edwards et al., 2009).



Staurosporine (R=H) / **UCN-01** (R=OH)



LY294002



Geldanamycin

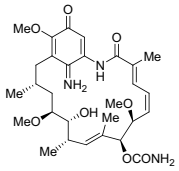


Figure 2. Fitness Factor Liabilities for Selected Early Chemical Probe Inhibitors of Protein Kinases, the PI3K Family of Lipid Kinases and the HSP90 Molecular Chaperone

Important fitness factors that have been improved significantly in subsequent chemical probes and clinical agents acting on these targets have been flagged. The symbols refer to the fitness factors listed in Figure 1, and the evolution of the probes to remove the liabilities shown is discussed in detail in the respective sections of the text.

Evolving Probes: One Size Does Not Fit All

We emphasize that the fitness factors in Figure 1 and the threshold values in Table 1 represent guidelines rather than proscriptive rules. Particularly when a molecular target or biological area is new, useful information can still be gained with a chemical probe that is not yet ideal (see examples later), and indeed may have obvious limitations with respect to fitness factors. Emerging probes will be road-tested by the biological community. They will embark on an evolutionary journey, struggling with other probes for survival. Unfit ones will be discarded whereas fit-for-purpose tools will be taken up and used. Improved probes will then emerge into the light and the chemical tools will evolve alongside the biological understanding.

It is clearly important that when new probes do emerge they are compared with the current best in class and that the added value is clear (Oprea et al., 2009). As mentioned, poor chemical probes can mislead biologists. But we also believe that excessive prescription will run counter to innovation. Potentially important probes in a new biological area must not be damned too quickly because they have a few rough edges. On the other hand, considering the fitness factors can help decide when a probe is fit-for-purpose, should encourage good practice and should avoid the worst examples that continue to contaminate the literature.

Examples of the sort of experience that has been gained with probing probes and evolving tools will be found in the oncology case histories discussed in the following sections. As an illustration, Figure 2 shows the limitations in fitness factors for selected early chemical probe inhibitors of the three classes of important targets that will be considered in detail, namely protein kinases, the PI3K family of lipid kinases, and the HSP90 molecular chaperone. In each case, the early probes were flawed but nevertheless proved useful, and the fitness factor profile was enhanced considerably during subsequent probe evolution, such that the liabilities were effectively removed.

Chemical Probes for Protein Kinase Inhibition

The close links between chemical biology and drug discovery are amply illustrated by the coevolution of protein kinase inhibitor tool compounds and pharmaceutical leads. Selectivity has been a key fitness factor consideration in protein kinase research, as will be highlighted.

Using and Improving Staurosporine

The natural product staurosporine (Tamaoki et al., 1986) (for the chemical structure of this and other selected protein kinase inhibitors, see Figure 3) has been a widely used probe of signal transduction biology in cells but has significant liabilities in its fitness factors (Figure 2). The compound and its analogs are now seen as unacceptably promiscuous (Bain et al., 2007; Frye, 2010). Very high binding to human plasma proteins was seen in clinical trials of the staurosporine analog UCN-01 (Fuse et al., 2005). Hypotheses based on the activities of the indolocarbazoles have nevertheless been productively investigated, and over time compounds with better utility have been developed (Nakano and Omura, 2009). The distinctive effect of the compounds on tumor cell cycle progression and apoptosis was originally interpreted as inhibition of protein kinase C (PKC), but it became apparent that a much wider range of protein kinases, or nonkinase targets, were inhibited (Akinaga et al., 2000; Prudhomme, 2003).

Substantial medicinal chemistry efforts were made to tease apart the activities of the indolocarbazole early leads (Roffey et al., 2009). For example, the bisindolylmaleimide ruboxistaurin (LY333351) was generated with somewhat improved selectivity for PKC (Jirousek et al., 1996; Bain et al., 2007). More recently, sotrastaurin (AEB071) has been reported as a potent and highly selective inhibitor of PKC isoforms (Wagner et al., 2009), as illustrated in the kinome profiles in Figure 4. Interestingly, the anilinopyrimidine scaffold that led ultimately to the BCL-ABL inhibitor imatinib, the first approved kinase-targeting anticancer drug, was discovered initially in a project to find new PKC inhibitors (Capdeville et al., 2002). It is also important to note that for structural biology studies involving wide comparisons across the kinase superfamily, the promiscuity of staurosporine has actually been exploited as a useful property (Tanramluk et al., 2009). In addition, probably because of its promiscuous effects, staurosporine is frequently used as a control compound in studies of apoptosis.

Elucidation of the multifactorial effects of the staurosporine analog UCN-01 on tumor cells led to an understanding of the role of the cell cycle regulator CHK1 (Senderowicz, 2000). UCN-01 and more selective inhibitors of checkpoint kinases, such as isogranulatimide (Jiang et al., 2004), abrogate the S

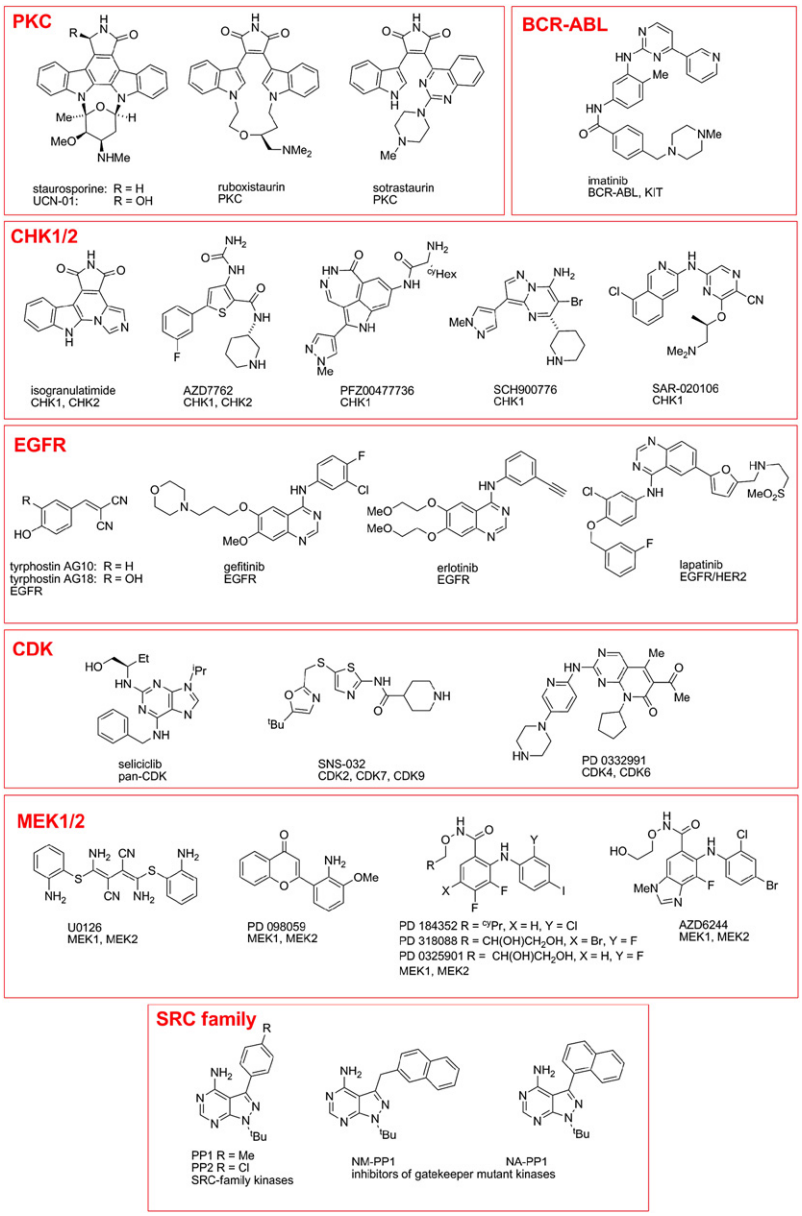


Figure 3. Structures of Selected Protein Kinase Chemical Probes Discussed in Detail in the Text with Their Main Proposed Targets Indicated

From Tyrphostins and Purines to Clinical Candidates

An early breakthrough in the development of chemical probes for kinases came with the rational design of the tyrphostin inhibitors of receptor tyrosine kinases (RTK) (Gazit et al., 1989). These compounds have poor chemistry fitness factors due to the presence of functional groups widely considered as undesirable, in particular polyphenolic residues associated with nonspecific protein binding and redox metabolism, as well as chemically reactive benzylidenemalononitriles. Some tyrphostins were later shown to uncouple the mitochondrial electron transport chain leading to ATP depletion and generalized inhibition of intracellular kinase signaling (Soltoff, 2004). Nevertheless, the tyrphostins were important pathfinder compounds that provided impetus for research into kinase inhibition, showing the ability to achieve selectivity at the ATP site, and leading to more refined chemical probes, as in the pioneering example of the epidermal growth factor (EGFR) RTK enzyme (Yaish et al., 1988; Levitski and Gazit, 1995). Following on, the anilinoquinazolines were an early, more drug-like scaffold providing stable and selective RTK inhibitors with much improved fitness factor profiles (Fry et al., 1994). This chemical class has since provided a plethora of excellent in vitro and in vivo probes and clinical candidates, including the approved drugs gefitinib, erlotinib, and lapatinib that show cellular and clinical activity in the context of genetic mutation or amplification of the EGFR and ERBB2 RTKs (Barker et al., 2001; Pollack et al., 1999; Xia et al., 2002).

A number of chemical probes for protein kinases evolved from structures inspired by the purine cofactor ATP (Vesely et al., 1994). The development of purine-derived inhibitors is an early example of the successful generation of novel probes through the application of combinatorial library synthesis combined with structural biology (Gray et al., 1998). Several purines, including seliciclib (*R*-roscovitine; Meijer et al., 1997) that is now in clinical trial for cancer (Whittaker et al., 2004; Benson et al., 2007), are appropriate probes for pan-cyclin dependent kinase (CDK) inhibition (Bain et al., 2007). The identification and refinement of other CDK inhibitor chemotypes has provided isoform-selective compounds, such as the pyridopyrimidinone CDK4/6-selective inhibitor PD 0332991 or the aminothiazole CDK2/7/9 inhibitor SNS-032 (Toogood et al., 2005; Misra et al., 2004). Importantly, the molecularly targeted drug discovery efforts again progress hand-in-hand with the evolution of better chemical probes.

and G2 checkpoints induced by DNA-damaging agents and thus potentiate their cytotoxicity. This exemplifies the use of multiple compounds to build confidence in the association of an effect to a particular target even where specificities are not high (Collins and Garrett, 2005). Selective inhibitors of CHK1, or dual inhibitors of CHK1 and CHK2, have now been developed and clinical trials are ongoing with agents including AZD7762, PFZ00477736, and SCH900776 (Bucher and Britten, 2008; Dai and Grant, 2010), whereas highly selective inhibitors of CHK1 have been identified as chemical probes with good performance across the fitness factors, e.g., SAR-020106 (Walton et al., 2010).

Thus it is clear that with all the limitations of staurosporine, it has provided the inspiration for a new generation of robustly fit-for-purpose probes with excellent fitness factor profiles for in vitro and in vivo use, as well as drugs in the clinic.

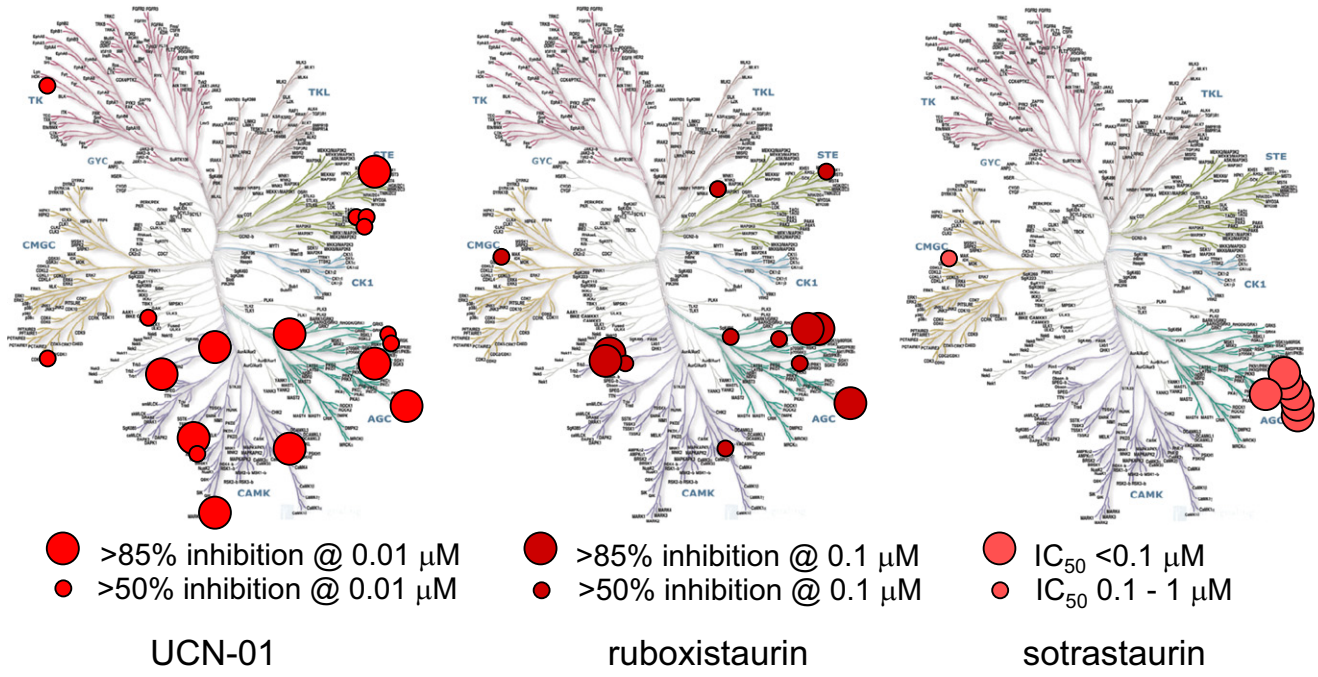


Figure 4. Evolution of Increasingly Selective PKC Inhibitors Based on Staurosporine
Percentage inhibitions for UCN-01 and ruboxistaurin were measured at 0.01 μM and 0.1 μM concentrations, respectively, for the same panel of 69 kinases (Bain et al., 2007). IC_{50} values were determined for sotrastaurin in a panel of 32 kinases (Wagner et al., 2009). The cluster of potent activities for sotrastaurin represents PKC isoform inhibition. All three compounds inhibit several PKC isoforms with IC_{50} 0.001–0.01 μM. Kinase dendrogram (Manning et al., 2002) reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com).

Beyond ATP Competition

To find highly selective chemical probes for kinases, there may be advantages in specifically targeting distinct inactive conformations of the ATP-binding site (Liu and Gray, 2006) or true allosteric modulatory sites, as demonstrated with selective AKT inhibitors (Cherrin et al., 2010).

The ATP noncompetitive inhibitor of MEK1/2 (MKK1/2), U0126, was identified from a cell-based screen for inhibitors of AP-1 mediated transcription (Duncia et al., 1998; Favata et al., 1998). The compound prevents activation of MEK1/2, leading to high potency in cells for inhibition of the RAF-MEK-ERK cascade. The 1,4-diamino-2,3-dicyanobutadiene moiety is an interconvertible mixture of steroisomers in solution, where activity resides uniquely in one isomer. Although unstable structures may be generally undesirable in chemical probes, the chemical behavior was reported and understood at an early stage, and U0126 has proved a useful chemical tool (Davies et al., 2000; Bain et al., 2007). HTS for other chemotypes of MEK ligand identified the chromenone PD 098059 as an alternative noncompetitive inhibitor with improved chemical fitness factors (Dudley et al., 1995), and MEK inhibitor development has led to enhanced, highly selective chemical probes and clinical candidates, including PD 184352, PD 0325901, and AZD6244 (ARRY142886) (Bain et al., 2007; Halilovic and Solit, 2008).

Structural biology studies with an analog of PD 184352 (PD 318088) showed the inhibitors to bind in an allosteric pocket adjacent to the ATP-binding site of MEK1/2, locking the enzymes in the unphosphorylated, inactive form (Ohren et al., 2004). The inhibitor PD 0325901 has been used as a cellular mechanistic probe to reveal biology context-dependent, selective therapeutic effects in that tumor cells driven by mutant BRAF have a unique vulnerability to MEK inhibition (Solit et al., 2006)—an effect also recapitulated recently both in cells and in the clinic with selective ATP-competitive BRAF inhibitors, whereas enhanced tumor progression is seen in cancers with NRAS mutations (Heidorn et al., 2010). Bain et al. (2007) recommend the use of PD 184352 or PD 0325901, with confirmation by the structurally unrelated U0126, as probes to inhibit MEK in cells.

Chemical Genetics and Kinase Inhibitors

An example of the development of protein kinase inhibitor chemical probes highlights the complementarity between chemical and genetic approaches. The pyrazolopyrimidines PP1 and PP2 were identified as inhibitors of SRC family kinases (Hanke et al., 1996). Structural studies showed the importance of a small gatekeeper residue and accessibility of the interior hydrophobic pocket of the kinase in determining the selectivity of the pyrazolopyrimidines (Liu et al., 1999). Exploiting this paradigm, mutant protein kinases where a large gatekeeper residue is replaced by alanine can be rendered sensitive to the pyrazolopyrimidines NM-PP1 and NA-PP1 that bear large substituents not generally tolerated by wild-type kinases (Bishop and Shokat, 1999). Transfection of cells with the drug-sensitized mutants thus allows highly selective, rapid, and reversible pharmacological inhibition of the target enzyme to be probed. However, NM-PP1 and NA-PP1 do inhibit some wild-type kinases and this should be taken into account in interpreting results from gatekeeper mutant proteins (Bain et al., 2007).

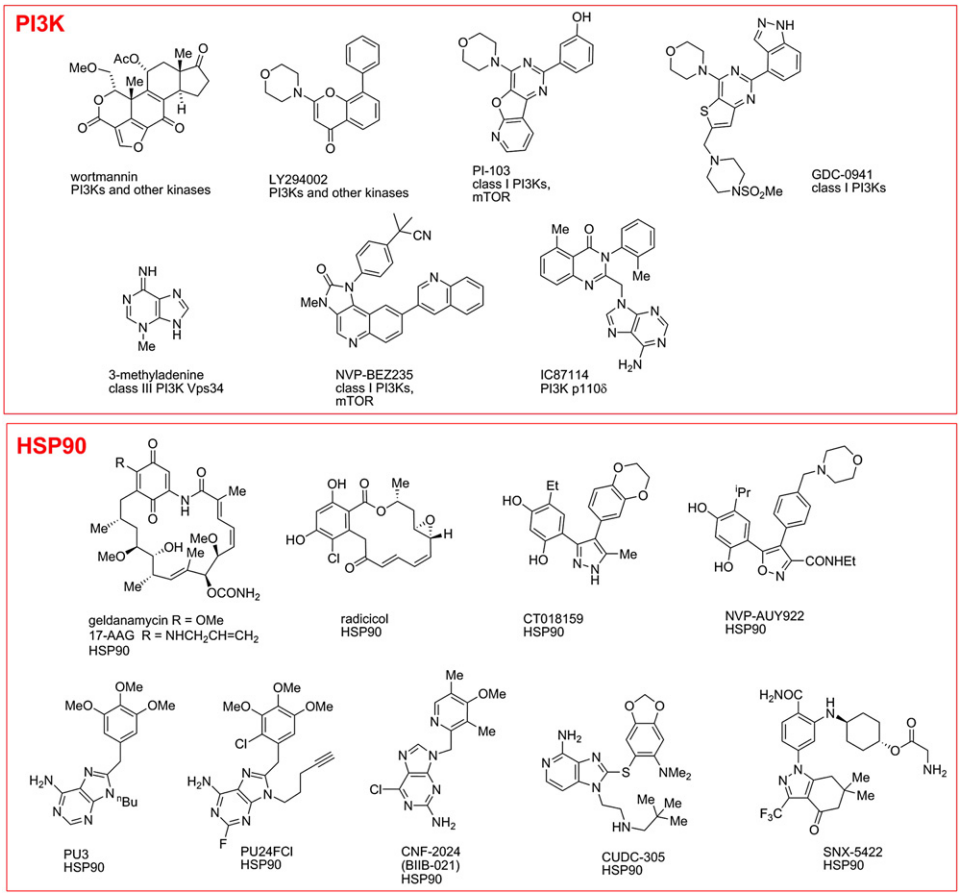


Figure 5. Structures of Selected PI3 Kinase and HSP90 Chemical Probes Discussed in Detail in the Text with Their Main Proposed Targets Indicated

In addition to inhibitor-sensitized mutants, drug-resistant mutant kinase alleles have also proved to be very useful (Brown et al., 1995; Eyers et al., 1999; Cohen, 2009) and are recommended for additional security as regards selectivity, alongside the use of two distinct chemotypes and inactive controls when interrogating kinase probe selectivity in cells (Cohen, 2009). For example, the cellular expression of BRAF alleles with mutant gatekeeper residues rendering them resistant to inhibition by small molecules has clearly shown that the multi-RTK inhibitor sorafenib does not exert its cellular antiproliferative effects through inhibition of BRAF, in contrast to the more recently developed inhibitor PLX4720 (Whittaker et al., 2010).

Protein kinase inhibitors provide arguably the best examples of context-dependent effects, with agents (such as imatinib in BCR-ABL positive or KIT mutant cancer cells, or the EGFR and ERBB2 inhibitors in cells with mutated and amplified targets) exhibiting selective antiproliferative and apoptotic outcomes in cells that have become "addicted" through, for example kinase mutation, amplification or translocation (Weinstein, 2002; Collins and Workman, 2006). Importantly, the same effects are also seen in the clinic.

Chemical Probes for PI3 Kinases

Phosphatidylinositide 3-kinases (PI3Ks) are key components of signal transduction pathways controlling a wide range of biological phenotypes and are deregulated in several diseases including immune inflammation and cancer. These lipid kinases phosphorylate the 3′-hydroxy position of the inositol ring of phosphoinositides (PI) generating PI second messengers downstream of RTKs and G protein coupled receptors. In parallel with biochemical and genetic studies (Cantley, 2002), our current understanding of the biological roles of PI3Ks has benefited enormously from the use of chemical probes over the last 20 years (Workman et al., 2010).

Flawed but Valuable Early PI3K Probes

Starting from probes that are now known to have significant limitations but which have proved useful in thousands of studies, these have evolved into more sophisticated chemical tools with much more attractive fitness factor profiles and also into the first PI3K drugs entering the clinic for cancer treatment (Workman et al., 2010).

The fungal furanosteroid metabolite wortmannin (see Figure 5 for the chemical structure of this and other selected PI3K inhibitors) was identified in 1987 as a potent inhibitor of the respiratory burst in neutrophils and monocytes (Baggiolini et al., 1987) and subsequently found to inhibit PI3K by covalent binding to a specific ATP site lysine (Wymann et al., 1996). LY294002, a synthetic chromone related to the bioflavonoid and broad spectrum protein kinase inhibitor quercetin, was discovered in

1994 by targeted screening of analogs using a biochemical assay and was found to inhibit PI3K competitively at the ATP site (Vlahos et al., 1994). Wortmannin is potent but unstable and was shown by profiling to inhibit smMLCK, PLK1, PI4K, and mTOR (Bain et al., 2007). Like wortmannin, LY294002 also has major defects in its fitness factors (Figure 2). It is only a weak PI3K inhibitor with a K_i of 1.6 μM, and activity in cells at 10–50 μM, and is also active against TORC1, CK2, PLK1, PIM1, PIM3, HIPK2, and GSK3, as well nonkinase off-targets, at concentrations similar to those that inhibit PI3K (Davies et al., 2000; Bain et al., 2007; Gharbi et al., 2007). Despite these limitations, wortmannin and LY294002 were used to generate invaluable understanding of physiological and pathological processes and to validate PI3K as a druggable target.

Evolving to Enhanced PI3K Probes and Drugs in the Clinic

Over the last few years, combinations of screening, medicinal chemistry, and structure-based design approaches have generated an exciting series of enhanced probes and drug candidates with markedly improved properties in respect of all of the fitness factors (Shuttleworth et al., 2009). A compound that is now recommended (Bain et al., 2007) as a chemical tool for class I PI3K/mTOR is the pyridofuropyrimidine PI-103 that has single digit nanomolar target potency, shows a high degree of selectivity against at least 70 kinases, and exhibits activity against animal models of cancer (Hayakawa et al., 2007; Raynaud et al., 2007). Optimization of PI-103 to reduce metabolic clearance and improve its pharmacokinetic properties resulted in the thienopyrimidine GDC-0941 that is now in Phase I clinical trials for cancer (Folkes et al., 2008; Raynaud et al., 2009). As with PI-103, detailed and quantitative molecular biomarker studies confirmed inhibition of the PI3K pathway in cells. GDC-0941 has the overall profile of a potent Class I PI3K inhibitor (acting at low nanomolar concentrations on all the class 1A isoforms p110α, p110β, and p110δ and the single class 1B isoform p110γ) with very high selectivity versus class II and III PI3K superfamily members. In contrast to PI-103, GDC-0941 also has low activity on the class IV PI3K superfamily protein kinases, including mTOR. Broader profiling showed that GDC-0941 is highly selective for PI3K with respect to a panel of 228 protein kinases. As would be required of a clinical candidate, GDC-0941 has negligible effects on CYP1A and CYP3A4 with no significant blockade of the HERG channel by patch clamp assay. A number of PI3K inhibitors are now in the clinic (Yap et al., 2008; Shuttleworth et al., 2009), including the imidazoquinoline NVP-BEZ235 that evolved by target hopping from a PDK1 inhibitor lead (Liu et al., 2009).

Understanding Selective PI3K Inhibition through Probes and Protein Structure

Obtaining selectivity among the Class I lipid kinases in the PI3K superfamily has been an important goal for probes and drugs. In a landmark chemical biology study, large numbers of PI3K inhibitors from journal articles and patents were synthesized and profiled against multiple enzymes (Knight et al., 2006), revealing intriguing cryptic homologies across PI3K targets and chemotypes. Clear selectivity trends were uncovered that were not predicted from the amino acid sequences. In terms of the use of these agents as chemical probes, the matrix of PI3K inhibitors was used to confirm the key role of p110α in insulin signaling, consistent with the mutant p110α mouse knock-in studies published around the same time (Foukas et al., 2006)—an example of chemical probe and genetic studies validating contemporaneously the same cutting edge biological result.

Of note, structural biology and modeling approaches have played a leading role in the design of PI3K inhibitors and in the interpretation of their potency and selectivity fitness factor properties (Workman et al., 2010). Pioneering X-ray crystal structures were obtained by Walker et al. (1999) for the apo and ATP-bound forms of p110γ and for early probes like wortmannin and LY294002 bound to this isoform (Walker et al., 2000). The X-ray structure of GDC-0941 explained its improved potency (Folkes et al., 2008). Very recently, structures of inhibitors complexed with the p110δ subunit have been determined, e.g., the quinazoline IC87114, which facilitated the design of new propeller-shaped compounds selective for the more conformationally flexible ATP site of this isoform (Berndt et al., 2010). Agents selective for p110δ and p110γ have potential in diseases such as immune inflammation and cancer, as well as being powerful chemical probes, including the p110δ clinical drug CAL-101 derived from IC87114 (Shuttleworth et al., 2009).

The very recently solved crystal structure of the class III PI3K Vps34, which is involved in autophagy, membrane trafficking and cell signaling, shows why it is so difficult to inhibit (Miller et al., 2010). A very weak Vps34 inhibitor, 3-methyladenine, has been used at 10 mM as a "specific" inhibitor of autophagy. New cocrystal structures of Vps34 with inhibitors have pointed the way to novel compounds with greater potency and selectivity over class I PI3Ks, highlighting the potential for probes with much better fitness factors for blocking Vps34 and autophagy in cells.

Using PI3K Probes

The testing in parallel of more than one probe chemotype, e.g., wortmannin plus LY294002 or PI-103 for class I lipid kinases, has been quite common in PI3K research. The application of companion inactive derivatives has been much less common, although early cellular work with LY294002 used analogs that were substituted in the essential morpholine ring to reduce hinge-binding (Vlahos et al., 1994). In terms of cellular context, cancer cells with mutations in the *PIK3CA* gene encoding p110α, or loss of the counteracting phosphatase PTEN, may be more sensitive to pan-class I selective PI3K inhibitors, whereas mutations in KRAS seem to confer resistance (Workman et al., 2010). As with protein kinases, the PI3K family is another excellent example of a target group for which the fitness factors of the probe compounds were initially less than we would now desire, but which were nevertheless sufficient to move the field forward markedly, leading through iterative improvements to the highly potent and selective probes that are available commercially and drugs that are now in the clinic.

Chemical Tools for the HSP90 Molecular Chaperone

HSP90 is a molecular chaperone that helps to control the stabilization and degradation of its "client" proteins, as well regulating their activated states (Workman et al., 2007). It has emerged as an exciting oncology target because inhibiting HSP90 causes depletion of multiple oncogenic clients, e.g., mutant kinases,

leading to blockade of many key cancer-causing pathways and the antagonism of the hallmark pathological traits of malignancy. Cancer selectivity is achieved by exploiting oncogene addiction as well as the stressed state of tumor cells (Workman et al., 2007).

Natural Product Origins of HSP90 Probes

HSP90 is an outstanding example of a contemporary pioneer drug target for which chemical probes played a leading role in the elucidation of the physiological and pathological functions of the protein, as well in establishing its druggability and reducing biological risk as perceived by industry. In particular the natural products geldanamycin and radicicol (Figure 5) were found in landmark studies in the 1990s to bind HSP90, specifically at its unusually shaped GHKL class of ATP-binding site, and thereby to inhibit the essential ATPase-driven chaperone cycle, resulting in client protein degradation (Whitesell et al., 1994; Roe et al., 1999). These valuable early natural product HSP90 probes turned out to be quite potent and selective for HSP90 with respect to the isolated biochemical target and also in cells. However, they nevertheless have other significant fitness factor limitations, particularly the metabolically labile quinone in geladanamycin (Figure 2) and the reactive epoxide group in radicicol, as well as other metabolism/stability issues, efflux pump and cytochrome P450 liabilities, and general toxicity problems.

Structure-Based Design of HSP90 Probes and Drugs

In parallel with the 17-allylamino analog of geldanamycin (17-AAG, tanespimicin) being developed for clinical evaluation, efforts were initiated to identify synthetic small molecule inhibitors that lacked the obvious undesirable features of the pathfinder natural products (see Figure 5 for the chemical structures of HSP90 inhibitors discussed in text). The purine PU3, designed by structure-based modeling, led the way; although showing only 15–20 μM potency on HSP90, it depleted specific chaperone clients in cancer cells, confirming an on-target mechanism (Chiosis et al., 2001). Optimization produced more potent purines, such as the in vivo active PU24FCl (Vilenchik et al., 2004) and the nanomolar potent, orally active clinical candidate BIIB021/CNF-2024 (Kasibhatla et al., 2007; Zhang et al., 2010).

The pyrazole/isoxazole resorcinol class of synthetic small molecule inhibitors was identified by biochemical screening. The original HTS hit CCT018159 was ATP-competitive with a K_d of 0.5 μM and, like PU3, caused client depletion in cancer cells; an indication of selectivity was shown by the relative lack of activity on the related GHKL class protein topoisomerase II and 20 protein kinases (Cheung et al., 2005; Sharp et al., 2007). X-ray crystallography confirmed a resorcinol-anchored binding mode identical to the natural product radicicol. Structure-based multiparameter optimization yielded the clinical candidate NVP-AUY922, now in Phase II trials (Brough et al., 2008; Eccles et al., 2008). This had a K_d of 2 nM, showed mechanism-based inhibition of cancer cell proliferation at ~9 nM and exhibited potent antitumor activity in animal models. A high level of selectivity for NVP-AUY922 was shown for the HSP90 α and β isoforms, as compared to the closely related HSP90 family chaperones GRP94 and TRAP-1, the GHKL superfamily member topoisomerase II and the structurally distinct molecular chaperone HSP72, as well as 13 representative kinases, 14 additional enzymes, and 67 receptors (Eccles et al., 2008).

A range of additional HSP90 inhibitory chemotypes have emerged recently (Biamonte et al., 2010), including clinical candidates like the pyrazolobenzamide SNX-5422 (Huang et al., 2009a) and the imidazopyridine CUDC-305/Debio 0932 (Bao et al., 2009).

Value of HSP90 Biomarkers Alongside Probes

The mutually enabling advances in basic, translational and drug discovery research on HSP90 have been facilitated especially by the combined use of chemical probes and mechanism-based molecular biomarkers. Early work identified the depletion of clients and activation of heat shock proteins as a direct result of HSP90 inhibition and these were also used to show target modulation by 17-AAG in cancer patients (Banerji et al., 2005). Further on-target versus off-target effects were revealed by comparing the unbiased global expression profiles for tumor cells treated with 17-AAG, an inactive analog and radicicol as an alternative active chemotype (Maloney et al., 2007). Interestingly, the upregulated gene products observed in that profiling study included members of the HSP70 family, HSP72 and HSC70, which have chaperone and antiapoptotic properties. This has led to these proteins being validated recently as cancer targets using RNAi together with several chemical probes of varying quality that collectively give some reassurance of potential druggability (Powers et al., 2008; Powers et al., 2010).

Chemical Tools for Other Oncology Targets–Supplemental Information

In the Supplemental Information available online, we highlight briefly a number of chemical probes (see Figure S1) for a range of additional oncology targets of different structural and functional types. These are selected as being of current interest to both basic and drug discovery research. They include p53 and BCL2 as good examples of important protein–protein interaction targets; chromatin-modifying enzymes, particularly histone deacetylases (see Figure S2), phosphatases; poly(ADP)ribose polymerase (PARP); and tankyrase. Some of them provide excellent additional examples of context-dependent biological effects of chemical probes and drugs, exploiting oncogene addiction and synthetic lethality in certain cancer cells in the context of particular mutation profiles.

Conclusions and Outlook

The field of chemical biology has matured in approach but is still growing fast in scale – and in a way that makes the emergence of guidelines for chemical probes almost inevitable. Given that analogous guidelines have been useful with fragments, leads and drugs in pharmaceutical discovery research, it seems likely that comparable guidance for probes will also be very valuable. Yet there are dangers. Taking deliberately extreme positions on this, the upside is that the following of sensible guidelines will result in probes that do what it says they should do on the tin and hence will prove to be useful research reagents; whereas the downside is that slavish adherence to a rule book could stifle innovation, particularly in the early stages of work on a new target or pathway for which probes are lacking.

We argue here that the correct strategy is somewhere in the middle. We recommend a common sense, fit-for-purpose approach and provide easy to use guidance on fitness factors for small molecule probes (Figure 1). These fitness factors

encompass four important areas—chemical properties, biological potency, biological selectivity, and context of use—that between them define the appropriateness of using a given chemical probe for exploratory biology. It is clearly not absolutely essential for all the requirements to be met at the level proposed by us and others. The case histories described here show that valuable progress can be made with initial probes that may well be suboptimal. At the same time, striving toward highly potent and selective cell permeable probes that are free of unnecessary chemical or biological baggage is highly desirable. Proponents of new probes and those who use them should be aware that suboptimal probes carry with them significant risk of off-target biological effects that may be general or context-dependent. The field should support transparency and availability of chemical probes so that the profiling and evidence-based refinement of these can proceed in an open source fashion by scientists around the world. At the same time, it is recognized that there is often a strong overlap between probes, leads and drugs and that some of this work will have to operate within the constraints that inevitably accompany pharmaceutical research if patients with serious diseases are to benefit from important new drugs.

The reality is that bad probes will bite the dust whereas better probes will evolve, thrive and prosper until they too are replaced by fitter and more powerful progeny—in a true Darwinian process. Improving the speed and efficiency of probe evolution will bring great benefits to basic, translational and drug discovery research as we move forward into the second decade of exploiting the human genome sequence.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures and Supplemental Text and can be found with this article online at doi:10.1016/j.chembiol.2010.05.013.

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WEB RESOURCES

www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm (accessed on 06.04.2010) The Drugs@FDA database provided by the FDA.

www.broadinstitute.org/genome_bio/connectivitymap.html (accessed on 06.04.2010). A detailed map generated at The Broad Institute that aims to link gene patterns associated with disease to corresponding patterns produced by drug candidates and a variety of genetic manipulations.

http://www.ebi.ac.uk/chembl/ (accessed on 28.03.2010). Website of the chEMBL team and access point to the chEMBLdb and kinase SARfari databases.

www.ensembl.org/info/about/species.html (accessed on 06.04.2010). The Ensembl project produces genome databases for vertebrates and other eukaryotic species, and makes this information freely available online.

http://investor.sigmaaldrich.com/releasedetail.cfm?releaseid=451709 (accessed on 07.05.2010). A press release describing plans to make certain chemical probes originated at Pfizer available for purchase.

http://mli.nih.gov/mli/wp-content/uploads/probe-guidelines-v2-accepted-by-sc-200707.doc (accessed on 28.03.2010). Probe Guidelines v2.0. Guidelines for chemical probes produced by the Molecular Libraries Probe Production Centers Network (MLPCN) of the NIH.

http://nihroadmap.nih.gov/molecularlibraries/index.asp (accessed on 06.04.2010). The website of the NIH Molecular Libraries Roadmap.

www.ornl.gov/sci/techresources/Human_Genome/home.shtml (accessed on 06.04.2010). The website of the Human Genome Project.

http://pubchem.ncbi.nlm.nih.gov/ (accessed on 5.04.2010). Database that provides information on the biological activities of small molecules; including substance information, compound structures, and BioActivity data in three primary databases, Pcsubstance, Pccompound, and PCBioAssay, respectively.

http://www.sanger.ac.uk/genetics/CGP/cosmic/ (accessed on 05.04.2010). Comprehensive source of somatic mutation information and related details and contains information relating to human cancers. The data can be queried by tissue, histology or gene and displayed as a graph, as a table or exported in various formats.

http://www.sanger.ac.uk/genetics/CGP/Translation (accessed on 16.06.2010). Entry page for the Cancer Translation Project that aims to screen >1000 genetically characterised human cancer cell lines with a wide range of anti-cancer therapeutics.

REFERENCES

Akinaga, S., Sugiyama, K., and Akiyama, T. (2000). Anticancer Drug Des. *15*, 43–52.

Amidon, G.L., Lennernäs, H., Shah, V.P., and Crison, J.R. (1995). Pharm. Res. *12*, 413–420.

Anonymous. (2009). Come together. Nat. Chem. Biol. *5*, 863.

Anonymous. (2010). Retooling chemical probes. Nat. Chem. Biol. *6*, 157.

Austin, C.P. (2003). Curr. Opin. Chem. Biol. *7*, 511–515.

Austin, C.P., Brady, L.S., Insel, T.R., and Collins, F.S. (2004). Science *306*, 1138–1139.

Baell, J.B., and Holloway, G.A. (2010). J. Med. Chem. *53*, 2719–2740.

Baggiolini, M., Dewald, B., Schnyder, J., Ruch, W., Cooper, P.H., and Payne, T.G. (1987). Exp. Cell Res. *169*, 408–418.

Bain, J., McLauchlan, H., Elliott, M., and Cohen, P. (2003). Biochem. J. *371*, 199–204.

Bain, J., Plater, L., Elliott, M., Shapiro, N., Hastie, C.J., McLauchlan, H., Klevernic, I., Arthur, J.S., Alessi, D.R., and Cohen, P. (2007). Biochem. J. *408*, 297–315.

Banerji, U., O'Donnell, A., Scurr, M., Pacey, S., Stapleton, S., Asad, Y., Simmons, L., Maloney, A., Raynaud, F., Campbell, M., et al. (2005). J. Clin. Oncol. *23*, 4152–4161.

Bao, R., Lai, C.J., Qu, H., Wang, D., Yin, L., Zifcak, B., Atoyan, R., Wang, J., Samson, M., Forrester, J., et al. (2009). Clin. Cancer Res. *15*, 4046–4057.

Bantscheff, M., Eberhard, D., Abraham, Y., Bastuck, S., Boesche, M., Hobson, S., Mathieson, T., Perrin, J., Raida, M., Rau, C., et al. (2007). Nat. Biotechnol. *25*, 1035–1044.

Barker, A.J., Gibson, K.H., Grundy, W., Godfrey, A.A., Barlow, J.J., Healy, M.P., Woodburn, J.R., Ashton, S.E., Curry, B.J., Scarlett, L., et al. (2001). Bioorg. Med. Chem. Lett. *11*, 1911–1914.

Benson, C., White, J., De Bono, J., O'Donnell, A., Raynaud, F., Cruickshank, C., McGrath, H., Walton, M., Workman, P., Kaye, S., et al. (2007). Br. J. Cancer *96*, 29–37.

Berndt, A., Miller, S., Williams, O., Le, D.D., Houseman, B.T., Pacold, J.I., Gorrec, F., Hon, W.C., Liu, Y., Rommel, C., et al. (2010). Nat. Chem. Biol. *6*, 117–124.

Berriman, M., Haas, B.J., LoVerde, P.T., Wilson, R.A., Dillon, G.P., Cerqueira, G.C., Mashiyama, S.T., Al-Lazikani, B., Andrade, L.F., Ashton, P.D., et al. (2009). Nature *460*, 352–358.

Biamonte, M.A., Van de Water, R., Arndt, J.W., Scannevin, R.H., Perret, D., and Lee, W.C. (2010). J. Med. Chem. *53*, 3–17.

Bishop, A.C., and Shokat, K.M. (1999). Pharmacol. Ther. *82*, 337–346.

Bishop, A.C., Ubersax, J.A., Petsch, D.T., Matheos, D.P., Gray, N.S., Blethrow, J., Shimizu, E., Tsien, J.Z., Schultz, P.G., Rose, M.D., et al. (2000). Nature *407*, 395–401.

Bologna, C. Promiscuity and Pubchem: A Retrospective Analysis. Proceedings of the Society for Biomolecular Screening 16th Annual Conference and Exhibition, Phoenix, Arizona, USA, April 11-15 2010, p119.

Brown, E.J., Beal, P.A., Keith, C.T., Chen, J., Shin, T.B., and Schreiber, S.L. (1995). Nature *377*, 441–446.

Brough, P.A., Aherne, W., Barril, X., Borgognoni, J., Boxall, K., Cansfield, J.E., Cheung, K.M., Collins, I., Davies, N.G., Drysdale, M.J., et al. (2008). J. Med. Chem. *51*, 196–218.

Bucher, N., and Britten, C.D. (2008). Br. J. Cancer *98*, 523–528.

Cantley, L.C. (2002). Science *296*, 1655–1657.

Capdeville, R., Buchdunger, E., Zimmermann, J., and Matter, A. (2002). Nat. Rev. Drug Discov. *1*, 493–502.

Cherrin, C., Haskell, K., Howell, B., Jones, R., Leander, K., Robinson, R., Watkins, A., Bilodeau, M., Hoffman, J., Sanderson, P., et al. (2010). Cancer Biol. Ther., in press. Published online April 4, 2010.

Cheung, K.M., Matthews, T.P., James, K., Rowlands, M.G., Boxall, K.J., Sharp, S.Y., Maloney, A., Roe, S.M., Prodromou, C., Pearl, L.H., et al. (2005). Bioorg. Med. Chem. Lett. *15*, 3338–3348.

Chiosis, G., Timaul, M.N., Lucas, B., Munster, P.N., Zheng, F.F., Sepp-Lorenzino, L., and Rosen, N. (2001). Chem. Biol. *8*, 289–299.

Clarke, P.A., Te Poele, R., Wooster, R., and Workman, P. (2001). Biochem. Pharmacol. *62*, 1311–1336.

Cohen, P. (2009). Biochem. J. *425*, 53–54.

Collins, F. (2010). Nature *464*, 674–675.

Collins, I., and Garrett, M.D. (2005). Curr. Opin. Pharmacol. *5*, 366–373.

Collins, I., and Workman, P. (2006). Nat. Chem. Biol. *2*, 689–700.

Dai, Y., and Grant, S. (2010). Clin. Cancer Res. *16*, 376–383.

Davies, S.P., Reddy, H., Caivano, M., and Cohen, P. (2000). Biochem. J. *351*, 95–105.

Davis, A.M., Keeling, D.J., Steele, J., Tomkinson, N.P., and Tinker, A.C. (2005). Curr. Top. Med. Chem. *5*, 421–439.

Dobson, C.M. (2004). Nature *432*, 824–828.

Dudley, D.T., Pang, L., Decker, S.J., Bridges, A.J., and Saltiel, A.R. (1995). Proc. Natl. Acad. Sci. USA *92*, 7686–7689.

Duncia, J.V., Santella, J.B., 3rd, Higley, C.A., Pitts, W.J., Wityak, J., Frietze, W.E., Rankin, F.W., Sun, J.H., Earl, R.A., Tabaka, A.C., et al. (1998). Bioorg. Med. Chem. Lett. *8*, 2839–2844.

Eccles, S.A., Massey, A., Raynaud, F.I., Sharp, S.Y., Box, G., Valenti, M., Patterson, L., de Haven Brandon, A., Gowan, S., Boxall, F., et al. (2008). Cancer Res. *68*, 2850–2860.

Edwards, A.M., Bountra, C., Kerr, D.J., and Willson, T.M. (2009). Nat. Chem. Biol. *5*, 436–440.

Emre, N., Coleman, R., and Ding, S. (2007). Curr. Opin. Chem. Biol. *11*, 252–258.

Entzeroth, M., Chapelain, B., Guilbert, J., and Hamon, V. (2000). J. Autom. Methods Manag. Chem. *22*, 171–173.

Eyers, P.A., van den IJssel, P., Quinlan, R.A., Goedert, M., and Cohen, P. (1999). FEBS Lett. *451*, 191–196.

Fabian, M.A., Biggs, W.H., 3rd, Treiber, D.K., Atteridge, C.E., Azimioara, M.D., Benedetti, M.G., Carter, T.A., Ciceri, P., Edeen, P.T., Floyd, M., et al. (2005). Nat. Biotechnol. *23*, 329–336.

Favata, M.F., Horiuchi, K.Y., Manos, E.J., Daulerio, A.J., Stradley, D.A., Feeser, W.S., Van Dyk, D.E., Pitts, W.J., Earl, R.A., Hobbs, F., et al. (1998). J. Biol. Chem. *273*, 18623–18632.

Frearson, J.A., and Collie, I.T. (2009). Drug Discov. Today *14*, 1150–1158.

Folkes, A.J., Ahmadi, K., Alderton, W.K., Alix, S., Baker, S.J., Box, G., Chuckowree, I.S., Clarke, P.A., Depledge, P., Eccles, S.A., et al. (2008). J. Med. Chem. *51*, 5522–5532.

Foukas, L.C., Claret, M., Pearce, W., Okkenhaug, K., Meek, S., Peskett, E., Sancho, S., Smith, A.J., Withers, D.J., and Vanhaesebroeck, B. (2006). Nature *441*, 366–370.

Fry, D.W., Kraker, A.J., McMichael, A., Ambroso, L.A., Nelson, J.M., Leoplod, W.R., Connors, R.W., and Bridges, A.J. (1994). Science *265*, 1093–1095.

Frye, S.V. (2010). Nat. Chem. Biol. *6*, 159–161.

Fuse, E., Kuwabara, T., Sparreboom, A., Sausville, E.A., and Figg, W.D. (2005). J. Clin. Pharmacol. *45*, 394–403.

Gazit, A., Yaish, P., Gilon, C., and Levitski, A. (1989). J. Med. Chem. *32*, 2344–2352.

Gharbi, S.I., Zvelebil, M.J., Shuttleworth, S.J., Hancox, T., Saghir, N., Timms, J.F., and Waterfield, M.D. (2007). Biochem. J. *404*, 15–21.

Golub, T. (2010). Nature *464*, 679.

Gray, N.S., Wodicka, L., Thunnissen, A.M., Norman, T.C., Kwon, S., Espinoza, F.H., Morgan, D.O., Barnes, G., LeClerc, S., Meijer, L., et al. (1998). Science *281*, 533–538.

Guillard, S., Clarke, P.A., Te Poele, R., Mohri, Z., Bjerke, L., Valenti, M., Raynaud, F., Eccles, S.A., and Workman, P. (2009). Cell Cycle *8*, 443–453.

Halilovic, E., and Solit, D.B. (2008). Curr. Opin. Pharmacol. *8*, 419–426.

Hanke, J.H., Gardner, J.P., Dow, R.L., Changelian, P.S., Brissette, W.H., Weringer, E.J., Pollok, B.A., and Connelly, P.A. (1996). J. Biol. Chem. *271*, 695–701.

Hayakawa, M., Kaizawa, H., Moritomo, H., Koizumi, T., Ohishi, T., Yamano, M., Ohta, M., Tsukamoto, S., Raynaud, F.I., Workman, P., et al. (2007). Bioorg. Med. Chem. Lett. *17*, 2438–2442.

Heidorn, S.J., Milagre, C., Whittaker, S., Nourry, A., Niculescu-Duvas, I., Dhomen, N., Hussain, J., Reis-Filho, J.S., Springer, C.J., Pritchard, C., et al. (2010). Cell *140*, 209–221.

Hoffmann, T., and Bishop, C. (2010). Drug Discov. Today *15*, 260–264.

Hopkins, A.L., and Groom, C.R. (2002). Nat. Rev. Drug Discov. *1*, 727–730.

Huang, K.H., Veal, J.M., Fadden, R.P., Rice, J.W., Eaves, J., Strachan, J.P., Barabasz, A.F., Foley, B.E., and Barta, T.E. (2009a). J. Med. Chem. *52*, 4288–4305.

Hughes, J.D., Blagg, J., Price, D.A., Bailey, S., Decrescenzo, G.A., Devraj, R.V., Ellsworth, E., Fobian, Y.M., Gibbs, M.E., Gilles, R.W., et al. (2008). Bioorg. Med. Chem. Lett. *18*, 4872–4875.

Huryn, D.M., and Cosford, N.D.P. (2007). Annu. Rep. Med. Chem. *42*, 401–416.

Inglese, J., Johnson, R.L., Simeonov, A., Xia, M., Zheng, W., Austin, C.P., and Auld, D.S. (2007). Nat. Chem. Biol. *3*, 466–479.

Jiang, X., Zhao, B., Britton, R., Lim, L.Y., Leong, D., Sanghera, J.S., Zhou, B.S., Piers, E., Andersen, R.J., and Roberge, M. (2004). Mol. Cancer Ther. *3*, 1221–1227.

Jirousek, M.R., Gillig, J.R., Gonzalez, C.M., Heath, W.F., McDonald, J.H., 3rd, Neel, D.A., Rito, C.J., Singh, U., Stramm, L.E., Melikian-Badalian, A., et al. (1996). J. Med. Chem. *39*, 2664–2671.

Kaiser, J. (2008). Science *321*, 764–766.

Kasibhatla, S.R., Hong, K., Biamonte, M.A., Busch, D.J., Karjian, P.L., Sensintaffar, J.L., Kamal, A., Lough, R.E., Brekken, J., Lundgren, K., et al. (2007). J. Med. Chem. *50*, 2767–2778.

Keiser, M.J., Setola, V., Irwin, J.J., Laggner, C., Abbas, A.I., Hufeisen, S.J., Jensen, N.H., Kuijer, M.B., Matos, R.C., Tran, T.B., et al. (2009). Nature *462*, 175–181.

Kerns, E.H., and Di, L. (2008). Drug-like properties: concepts, structure design and methods: from ADME to toxicity optimization (London: Academic Press).

Knight, Z.A., Gonzalez, B., Feldman, M.E., Zunder, E.R., Goldenberg, D.D., Williams, O., Loewith, R., Stokoe, D., Balla, A., Toth, B., et al. (2006). Cell *125*, 733–747.

Kodadek, T. (2010). Nat. Chem. Biol. *6*, 162–165.

Lamb, J., Crawford, E.D., Peck, D., Modell, J.W., Blat, I.C., Wrobel, M.J., Lerner, J., Brunet, J.P., Subramanian, A., Ross, K.N., et al. (2006). Science *313*, 1929–1935.

Lander, E.S., Linton, L.M., Birren, B., Nusbaum, C., Zody, M.C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., et al. (2001). Nature *409*, 860–921.

Lazo, J.S., Brady, L.S., and Dingledine, R. (2007). Mol. Pharmacol. *72*, 1–7.

Levitski, A., and Gazit, C. (1995). Science *267*, 1782–1788.

Lipinski, C.A. (2003). Compound properties and drug quality. In The Practice of Medicinal Chemistry, Second Edition, C.G. Wermuth, ed. (London: Academic Press), p. 341.

Lipinski, C.A., Lombardo, F., Dominy, B.W., and Feeney, P.J. (2001). Adv. Drug Deliv. Rev. *46*, 3–26.

Liu, Y., and Gray, N. (2006). Nat. Chem. Biol. *2*, 358–364.

Liu, Y., Bishop, A., Witucki, L., Kraybill, B., Shimuza, E., Tsien, J., Ubersax, J., Blethrow, J., Morgan, D.O., and Shokat, K.M. (1999). Chem. Biol. *6*, 671–678.

Liu, T.J., Koul, D., LaFortune, T., Tiao, N., Shen, R.J., Maira, S.M., Garcia-Echevrria, C., and Yung, W.K. (2009). Mol. Cancer Ther. *8*, 2204–2210.

Maloney, A., Clarke, P.A., Naaby-Hansen, S., Stein, R., Koopman, J.O., Akpan, A., Yang, A., Zvelebil, M., Cramer, R., Stimson, L., et al. (2007). Cancer Res. *67*, 3239–3253.

Manning, G., Whyte, D.B., Martinez, R., Hunter, T., and Sudarsanam, S. (2002). Science *298*, 1912–1934.

McGovern, S.L., Helfand, B.T., Feng, B., and Shoichet, B.K. (2003). J. Med. Chem. *46*, 4265–4272.

Meijer, L., Borgne, A., Mulner, O., Chong, J.P., Blow, J.J., Inagaki, N., Inagaki, M., Delcros, J.G., and Moulinoux, J.P. (1997). Eur. J. Biochem. *243*, 527–536.

Miller, S., Tavshanjian, B., Oleksy, A., Perisic, O., Houseman, B.T., Shokat, K.M., and Williams, R.L. (2010). Science *327*, 1638–1642.

Misra, R.N., Xiao, H.Y., Kim, K.S., Lu, S., Han, W.C., Barbosa, S.A., Hunt, J.T., Rawlins, D.B., Shan, W., Ahmed, S.Z., et al. (2004). J. Med. Chem. *47*, 1719–1728.

Nakano, H., and Omura, S. (2009). J. Antibiot. (Tokyo) *62*, 17–26.

Ohren, J.F., Chen, H., Pavlovsky, A., Whitehead, C., Zhang, E., Kuffa, P., Yan, C., McConnell, P., Spessard, C., Banotai, C., et al. (2004). Nat. Struct. Mol. Biol. *11*, 1192–1197.

Oprea, T.I., Allu, T.K., Fara, D.C., Rad, R.F., Ostopvici, L., and Bologa, C.G. (2007). J. Comput. Aided Mol. Des. *21*, 113–119.

Oprea, T.I., Bologa, C.G., Boyer, S., Curpan, R.F., Glen, R.C., Hopkins, A.L., Lipinski, C.A., Marshall, G.R., Martin, Y.C., Ostopovici-Halip, L., et al. (2009). Nat. Chem. Biol. *5*, 441–447.

Oprea, T.I., Davis, A.M., Teague, S.J., and Leeson, P.D. (2001). J. Chem. Inf. Comput. Sci. *41*, 1308–1315.

Overington, J.P., Al-Lazikani, B., and Hopkins, A.L. (2006). Nat. Rev. Drug Discov. *5*, 993–996.

Petri Seiler, K., Kuehn, H., Pat Happ, M., Decaprio, D., and Clemons, P.A. (2008). Curr. Protoc. Bioinformatics. Chapter 14:Unit 14.5.

Pollack, V.A., Savage, D.M., Baker, D.A., Tsaparikos, K.E., Sloan, D.E., Moyer, J.D., Barbacci, E.G., Pustilnik, L.R., Smolarek, T.A., Davis, J.A., et al. (1999). J. Pharmacol. Exp. Ther. *291*, 739–748.

Powers, M.V., Clarke, P.A., and Workman, P. (2008). Cancer Cell *14*, 250–262.

Powers, M.V., Jones, K., Barillari, C., Westwood, I., van Montfort, R.L.M., and Workman, P. (2010). Cell Cycle *9*, 1542–1550.

Price, D.A., Blagg, J., Jones, L., Greene, N., and Wager, T. (2009). Expert Opin. Drug Metab. Toxicol. *5*, 921–931.

Prudhomme, M. (2003). Eur. J. Med. Chem. *38*, 123–140.

Raynaud, F.I., Eccles, S., Clarke, P.A., Hayes, A., Nutley, B., Alix, S., Henley, A., Di-Stefano, F., Ahmad, Z., Guillard, S., et al. (2007). Cancer Res. *67*, 5840–5850.

Raynaud, F.I., Eccles, S.A., Patel, S., Alix, S., Box, G., Chuckowree, I., Folkes, A., Gowan, S., De Haven Brandon, A., Di Stefano, F., et al. (2009). Mol. Cancer Ther. *8*, 1725–1738.

Rees, D.C., Congreve, M., Murray, C.W., and Carr, R.A.E. (2004). Nat. Rev. Drug Discov. *3*, 660–672.

Rishton, G.M. (2003). Drug Discov. Today *8*, 86–96.

Roe, S.M., Prodromou, C., O'Brien, R., Ladbury, J.E., Piper, P.W., and Pearl, L.H. (1999). J. Med. Chem. *42*, 260–266.

Roffey, J., Rosse, C., Linch, M., Hibbert, A., McDonald, N.Q., and Parker, P.J. (2009). Curr. Opin. Cell Biol. *21*, 268–279.

Schreiber, S.L. (1998). Bioorg. Med. Chem. *6*, 1127–1152.

Schreiber, S.L. (2003). Chem. Eng. News *81*, 51–61.

Senderowicz, A.M. (2000). Oncogene *19*, 6600–6606.

Sharma, S.V., Haber, D.A., and Settleman, J. (2010). Nat. Rev. Cancer *10*, 241–253.

Sharp, S.Y., Boxall, K., Rowlands, M., Prodromou, C., Roe, S.M., Maloney, A., Powers, M., Clarke, P.A., Box, G., Sanderson, S., et al. (2007). Cancer Res. *67*, 2206–2216.

Shuttleworth, S., Silva, F., Tomassi, C., Cecil, A., Hill, T., Rogers, H., and Townsend, P. (2009). Progress in the design of phosphoinositide 3-kinase inhibitors for the treatment of chronic disorders. In Progress in Medicinal Chemistry, Volume 48, G. Lawton and D.R. Witty, eds. (New York: Elsevier), pp. 81–131.

Smyth, L.A., and Collins, I. (2009). J. Chem. Biol. *2*, 131–151.

Solit, D.B., Garraway, L.A., Pratilas, C.A., Sawai, A., Getz, G., Basso, A., Ye, Q., Lobo, J.M., She, Y., Osman, I., et al. (2006). Nature *439*, 358–362.

Soltoff, S.P. (2004). J. Biol. Chem. *279*, 10910–10918.

Sperandio, O., Reynès, C.H., Camproux, A.C., and Villoutreix, B.O. (2010). Drug Discov. Today *15*, 220–229.

Stratton, M.R., Campbell, P.J., and Futreal, P.A. (2009). The cancer genome. Nature *458*, 719–724.

Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M., and Tomita, F. (1986). Biochem. Biophys. Res. Commun. *135*, 397–402.

Tanramluk, D., Schreyer, A., Pitt, W.R., and Blundell, T.L. (2009). Chem. Biol. Drug Des. *74*, 16–24.

Tolliday, N., Clemons, P.A., Ferraiolo, P., Koehler, A.N., Lewis, T.A., Li, X., Schreiber, S.L., Gerhard, D.S., and Eliasof, S. (2006). Cancer Res. *66*, 8935–8942.

Toogood, P.L., Harvey, P.J., Repine, J.T., Sheehan, D.J., VanderWel, S.N., Zhou, H., Keller, P.R., McNamara, D.J., Sherry, D., Zhu, T., et al. (2005). J. Med. Chem. *48*, 2388–2406.

van der Waterbeemd, H. (2002). Physicochemical properties. In Medicinal Chemistry Principles and Practice, Second Edition, F. King, ed. (Cambridge, UK: The Royal Society of Chemistry), p. 195.

Venter, J.C., Adams, M.D., Myers, E.W., Li, P.W., Mural, R.J., Sutton, G.G., Smith, H.O., Yandell, M., Evans, C.A., Holt, R.A., et al. (2001). Science *291*, 1304–1351.

Vesely, J., Havlicek, L., Strnad, M., Blow, J.J., Donella-Deana, A., Pinna, L., Letham, D.S., Kato, J., Detivaud, L., Leclerc, S., and Meijer, L. (1994). Eur. J. Biochem. *224*, 771–786.

Vilenchik, M., Solit, D., Basso, A., Huezo, H., Lucas, B., He, H., Rosen, N., Spampinato, C., Modrich, P., and Chiosis, G. (2004). Chem. Biol. *11*, 787–797.

Vlahos, C.J., Matter, W.F., and Hui, K.Y. (1994). J. Biol. Chem. *269*, 5241–5248.

Wagner, J., von Matt, P., Sedrani, R., Albert, R., Cooke, N., Ehrhardt, C., Geiser, M., Rummel, G., Stark, W., Strauss, A., et al. (2009). J. Med. Chem. *52*, 6193–6196.

Walker, E.H., Pacold, M.E., Perisic, O., Stephens, L., Hawkins, P.T., Wymann, M.P., and Williams, R.L. (2000). Mol. Cell *6*, 909–919.

Walker, E.H., Perisic, O., Ried, C., Stephens, L., and Williams, R.L. (1999). Nature *402*, 313–320.

Walton, M.I., Eve, P.D., Hayes, A., Valenti, M., De Haven Brandon, A., Box, G., Boxall, K.J., Aherne, G.W., Eccles, S.A., Raynaud, F.I., et al. (2010). Mol. Cancer Ther. *9*, 89–100.

Weinstein, I.B. (2002). Science *297*, 63–64.

Weiss, W.A., Taylor, S.S., and Shokat, K.M. (2007). Nat. Chem. Biol. *3*, 739–744.

Whitesell, L., Mimnaugh, E.G., De Costa, B., Myers, C.E., and Neckers, L.M. (1994). Proc. Natl. Acad. Sci. USA *91*, 8324–8328.

Whittaker, S., Kirk, R., Hayward, R., Zambon, A., Viros, A., Cantarino, N., Affolter, A., Nourry, A., Niculescu-Duvaz, D., Springer, C., et al. (2010). Sci. Transl. Med. *2*, 35–41.

Whittaker, S.R., Walton, M.I., Garrett, M.D., and Workman, P. (2004). Cancer Res. *64*, 262–272.

Wohnsland, F., and Faller, B. (2001). J. Med. Chem. *44*, 923–930.

Workman, P. (2003). Curr. Pharm. Des. *9*, 891–902.

Workman, P., Burrows, F., Neckers, L., and Rosen, N. (2007). Ann. N Y Acad. Sci. *1113*, 202–216.

Workman, P., Clarke, P.A., Raynaud, F.I., and van Montfort, R.L. (2010). Cancer Res. *70*, 2146–2157.

Wymann, M.P., Bulgarelli-Leva, G., Zvelebil, M.J., Pirola, L., Vanhaesebroeck, B., Waterfield, M.D., and Panayotou, G. (1996). Mol. Cell. Biol. *16*, 1722–1733.

Xia, W., Mullin, R.J., Keith, B.R., Liu, L.H., Ma, H., Rusnak, D.W., Owens, G., Alligood, K.J., and Spector, N.L. (2002). Oncogene *21*, 6255–6263.

Yaish, P., Gazit, A., Gilon, C., and Levitski, A. (1988). Science *242*, 933–935.

Yap, T.A., Garrett, M.D., Walton, M.I., Raynaud, F., de Bono, J.S., and Workman, P. (2008). Curr. Opin. Pharmacol. *8*, 393–412.

Yap, T.A., Sandhu, S.K., Workman, P., and de Bono, J.S. (2010). Nat. Rev. Cancer, advance online publication, Published online 10 June 2010 | doi:10.1038/nrc2870.

Zhang, H., Neely, L., Lundgren, K., Yang, Y.C., Lough, R., Timple, N., and Burrows, F. (2010). J. Cancer *126*, 1226–1234.