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Targeting signal transduction with large combinatorial collections

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The large-scale application of combinatorial chemistry to drug discovery is an endeavor that is now more than ten years old. The growth of chemical libraries together with the influx of novel genomic targets has led to a reconstruction of the drug-screening paradigm. The drug discovery industry faces a post-genomic world where the interplay between tens-of-thousands of proteins must be addressed. To compound this complexity, there now exists the ability to screen millions of compounds against a single target. This review focuses on the practice and use of selecting individual compounds from large chemical libraries that act on targets relevant to signal transduction.

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▼ The majority of marketed drugs function through molecular targets operating in signal transduction pathways. Recently, crude roadmaps for some of the well-studied pathways have been presented and reviewed [1]. These pathways are not simple linear progressions but involve a significant amount of cross-talk. This network of biochemical interactions mediates the life of a cell from cellular growth and differentiation to apoptosis. Therefore, these molecular components have been a rich source of drug targets [2].

The most popular signal transduction targets in drug discovery have been the heterotrimeric GTP-binding G-protein-coupled receptors (GPCRs). Over the past 130 years of drug discovery, ~500 molecular targets have been identified and nearly 45% of these are GPCRs [3]. These include modulators of GPCRs, whose natural ligands are small molecules (e.g. histamine, serotonin and β-adrenergic receptors) and peptides (e.g. opioids). Part of the success of GPCRs in drug discovery is because they are expressed at the cell surface and thus the drug does not have to penetrate and function inside the cell. In addition, GPCR–ligand interactions can also be effected through a variety of mechanisms because these receptors exist in both agonist and antagonist conformations that are stabilized by ligands and specific G-proteins [4–5]. Small-molecule drugs could therefore act as agonists by providing structural mimics of the natural ligands that mimic the peptidic 'message sequence' (e.g. morphine, which mimics the peptidic sequence found in opioid peptides), and antagonists can mimic a characteristic 'address sequence' [6]. In many cases, the cognate ligand of a GPCR is a small molecule, although GPCRs can provide additional sites for allosteric modulation, for example, benzodiazepine tranquilizers, which effect the GABA receptors [7]. Several pharmaceutical companies have used combinatorial chemistry to generate surrogate ligands for orphan GPCRs [8,9].

Selectivity among GPCRs is often an issue, analogous to many target classes that are promiscuous and abundant in nature. Combinatorial chemistry can provide thousands of analogs based around a common core structure, which is often necessary to produce selective structures. This was exemplified in a study that screened a 150,000-member combinatorial library against CXCR2 (IL8 receptor) and the bradykinin 1 (BK1) receptor [10]. The library had four positions of variation (R groups) based around a common core structure, and was constructed with 31 substituents at R1 and R2, four at R3 and 38 at R4. Each receptor exhibited an active hit-rate within this library of <0.01% and selected only a few substituents at each position. For example, the CXCR2 receptor selected a single substituent at three of the four R groups, and the BK1 receptor selected a different set of substituents. Therefore, clear structural differences were observed between the set of compounds active at either CXCR2 or BK1.

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Although derived from the same library, small variations in the compounds provided structures for each GPCR that occupied unique regions of chemical diversity and were therefore highly selective (>100-fold against a panel of 50 molecular targets).

Protein–protein interactions such as the regulation of tyrosine kinases by SH2 and SH3 domains are also abundant in signal transduction pathways, although there has been no published successes of drugs that effect signal transduction through these interactions. To date, inhibitors of SH2 domains are either phosphomimetics or peptidomimetics [11–13]. In contrast to the GPCR target class, the majority of protein–protein interactions occur within the cell. However, the physicochemical properties of these mimetic compounds are not compatible with cell penetration and hence these compounds often have poor efficacy and oral bioavailability. The lack of small-molecule inhibitors for domain–domain interactions could stem from the dynamics of the binding site, which is often large and involves many weak interactions. The site is not optimized for potency but rather simply provides a potency that is adequate for a particular cellular pathway. A small-molecule drug must therefore find new interactions within this site to compete effectively with natural ligands [14].

The switches capable of turning cellular signals up or down often involve kinases and phosphatases, and combinatorial chemistry has provided several selective small-molecule inhibitors of these enzymes [12,15–17]. The majority of structures used in kinase discovery programs are based on ATP-competitive scaffolds [13], and selective molecules can be found despite the relatively high conservation of the kinase ATP-binding domain. Selectivity can be achieved by binding to idiosyncratic pockets not used by ATP, as well as by stabilizing the 'inactive' conformation of the kinase. The success of the Bcr-Abl inhibitor Gleevec (formerly ST-571) [18] is an example of a relatively selective tyrosine kinase inhibitor that stabilizes the inactive kinase conformation. This drug was discovered through the combination of combinatorial chemistry with HTS. Similar progress has been made with the serine and threonine class of kinases. Selective mitogen-activated kinase p38 inhibitors that disrupt cytokine signaling in cells were also identified through combinatorial chemistry and HTS [19,20]. Recently, the phosphatase PTP1B has been subjected to a number of HTS activities owing to its role in insulin resistance. A virtual screen of a 150,000-member library using the crystal structure of PTP1B bound to pTyr identified compounds with activity against this phosphatase [21]. Similar approaches have been used for the design and optimization of combinatorial libraries directed at kinases [22,23].

Case study of a combinatorial collection

Target class success

Since 1992, more than 1250 combinatorial libraries have been described from both academic and industrial laboratories [24]. Typically at Pharmacopeia (http://www.pharmacopeia.com), more than four million compounds are screened per target from a total collection of seven million compounds. This collection of encoded combinatorial libraries was made following a solid-phase binary-encoding protocol that uses electrophoric molecular tags (ECLiPs™ technology) and direct-divide synthesis [25–28]. Figure 1a summarizes the activity of Pharmacopeia's screening collection against more than 70 molecular targets. The two most widely screened classes of signal transduction targets have been GPCRs and kinases (Fig. 1). More than 50% of the targets screened in these two classes yielded active compounds with potencies of <1 μM. Cell-signaling assays using cell-based assays have also yielded viable compounds, although, in many cases, the molecular target remains unidentified. One exception to this is the successful use of an HTS assay based on nitric-oxide production from human cells, which led to the identification of a class of pyrimidineimidazole compounds that bind to and inhibit human inducible nitric oxide synthase (iNOS) [29,30]. However, to date, no compounds have been identified that

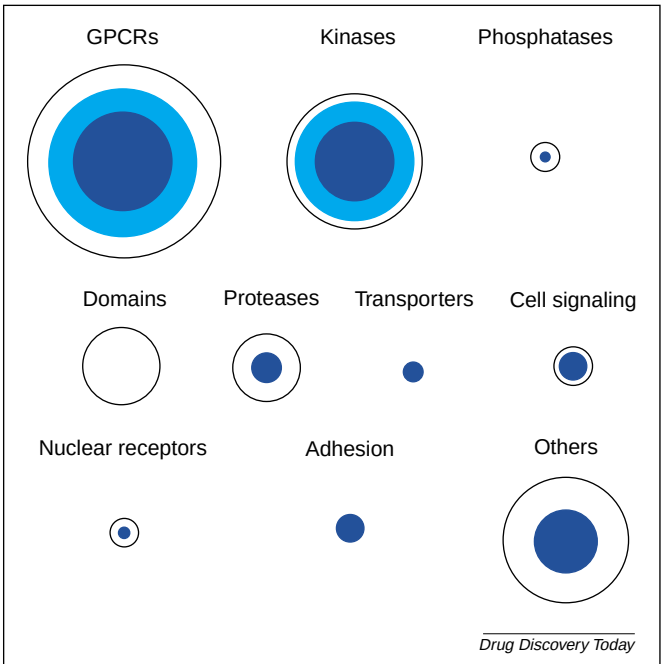

Figure 1. Target class success. The size of each circle is representative of the number of targets screened for each class. The dark and light blue circles represent the number of targets where active compounds were confirmed with <1 μM or <5 μM potency, respectively.

inhibit domain–domain interactions (Fig. 1). As the current compound collection at Pharmacopeia is non-peptidic in nature, the peptidomimetics mentioned above are not present in the collection. The active compounds summarized in Fig. 1 were identified in random screening campaigns, with the exception of proteases where focused libraries were made.

Constructing drug-like libraries

Potency for a biological target is only one of the desirable qualities of a combinatorial library. The marriage of combinatorial chemistry and HTS has significantly increased the number of leads found in drug screening programs. However, to date, there are no published examples of approved drugs borne of this combination. The reason for this could be attributed to the relative novelty of the field; many compounds that have entered the clinic might be approved in the near future [31]. Indeed, the first report of a compound derived from a solid-phase discovery library that has been tested in humans was recently reported by Ontogen (http://www.ontogen.com) for a P-glycoprotein modulator [32,33]. In addition, Gleevec was launched to Phase IV trials in the spring of 2001 [34], and the CCR1 receptor antagonist, also discovered using HTS (Berlex; http://berlex.com), has entered Phase I trials [35,36]. Several computational models have now been developed to predict the drug-likeness of a compound [37–39], thereby facilitating the progression of leads to drugs.

The goal of many drug discovery programs is to design a drug with oral bioavailability. This is determined by the ADME (absorption, distribution, metabolism and excretion) properties of the drug. Many models have now been developed that attempt to predict the oral bioavailability of a molecule based on its physicochemical properties [40,41]. GlaxoSmithKline (http://www.gsk.com) have developed a model based on the number of rotatable bonds and polar surface area (PSA) to assess the potential oral bioavailability of a drug [42]. Lipinski's rule-of-five is a model of passive intestinal absorption and solubility that has been of particular interest [43]. This model is based on the examination of 2287 compounds that have passed Phase I clinical trails, and states that chemical matter will be less likely to be permeable and soluble if cLogP is >5, molecular weight (MW) is >500, or if the number of H-bond donors or acceptors are >5 or >10, respectively. Figure 2a gives the results of an analysis of 4.2 million compounds derived from the Pharmacopeia compound collection using Lipinski's rule-of-five. The compounds in this collection are from libraries whose design involved filtering substituents *in silico* for rule-of-five violations. A model of passive intestinal absorption has also been developed at Pharmacopeia [44] based on a statistical pattern-recognition model using a panel of well-absorbed compounds (>90% absorbed) and poorly absorbed compounds (<30% absorbed). This model was used with Pharmacopeia's screening collection, and the results are shown in Fig. 2b. In this model, the calculated lipophilicity of the molecule (AlogP98 [45]) and the PSA are used to describe the physicochemical parameters. The inner ellipse represents the 95% confidence region for well-absorbed compounds while the outer ellipse represents the 99% confidence region. The figure shows that 49% of the collection fall within the 95% confidence ellipse and 67% fall within the 99% ellipse. These figures compare with the analysis of two other databases composed of drug-like chemical matter: ~100,000 compounds from the MACCS-II Drug Data Report yielded 64% and 76% inside the 95% and 99% ellipses, respectively (Fig. 2c); and 6000 compounds from the Comprehensive Medicinal Chemistry database (CMC) yielded 75.0% and 83.5% inside the 95% and 99% ellipses, respectively (Fig. 2d). This model enables the straightforward evaluation of compounds because it based on two readily calculated parameters.

An alternative approach to combinatorial library design centers on making 'lead-like' compounds rather than 'drug-like' compounds and uses a rule-of-three model (a molecular weight range of 100–300 Da and a calculated log P of 1–3 [46]). This is based on the fact that optimizing library hits for improved potency, selectivity and pharmacokinetics often involves increasing the molecular weight and lipophilicity of the molecule. Therefore, starting with smaller leads facilitates the selection of an optimized drug-like molecule. In addition, as noted by Hann [47], the complexity of a combinatorial library should not be solely restricted to drug-like properties because this might decrease the chance of identifying initial hits from the collection. Incorporating the afore-mentioned approaches into library design should provide hits with good initial solubility and absorption so that they can advance into lead optimization programs.

Mining combinatorial libraries

Random screening of this diverse collection of compounds has revealed three possible outcomes for a combinatorial library. After synthesis and screening, the library might fail to yield any hits against the target panel, in which case it remains an orphan. For active libraries screened across a panel of different molecular targets, two possible activity patterns can be seen: the library will either exhibit a preference for one particular target class (e.g. kinases) or show hits across different target classes. The latter is said to contain 'privileged' structures, a term initially introduced by Evans

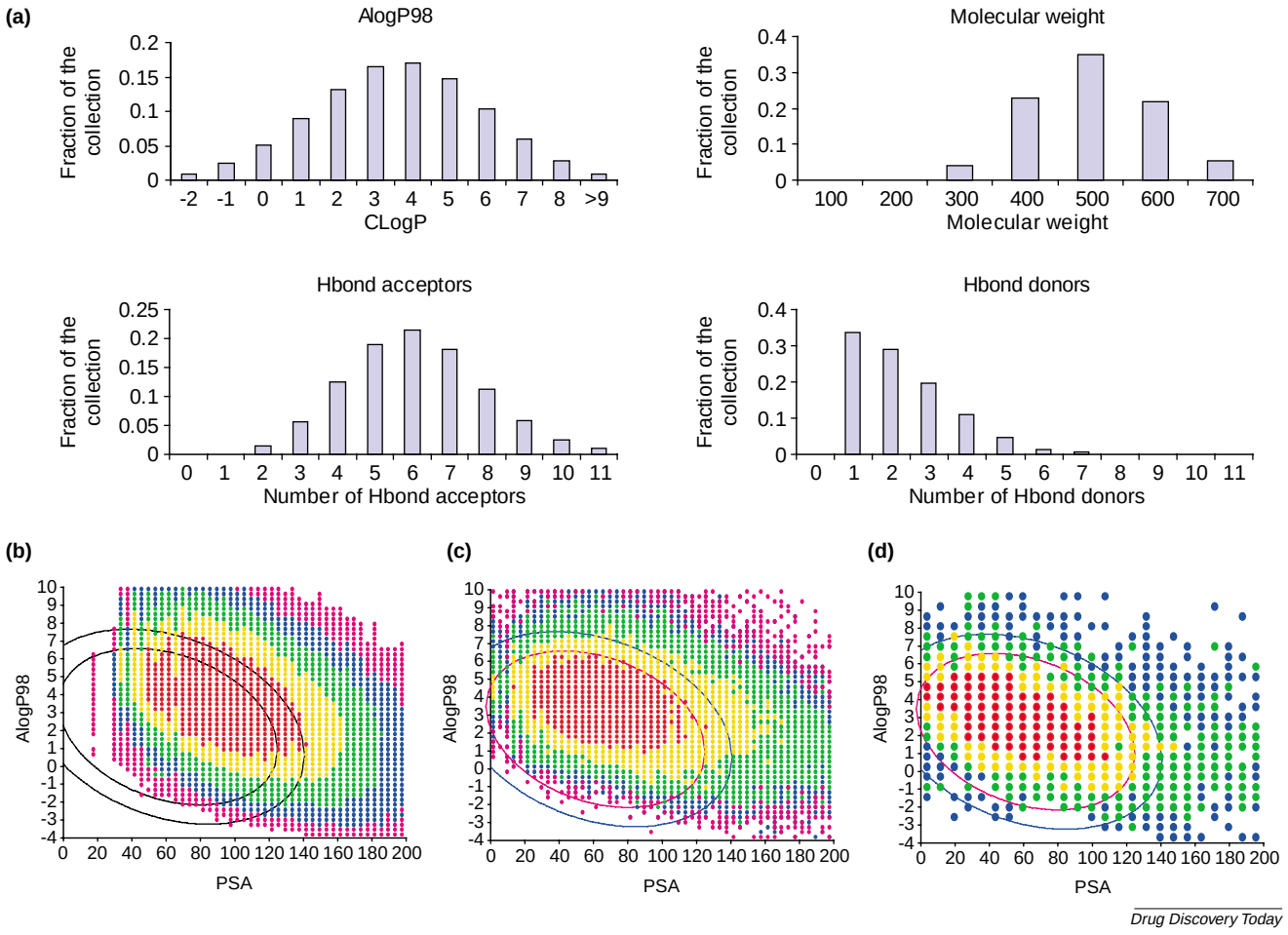

Figure 2. (a) Lipinski analysis of 4.2×10^6 combinatorial compounds from the Pharmacopeia collection (http://www.pharmacopeia.com). Model of absorption is shown for **(b)** the Pharmacopeia collection, **(c)** the MACCS-II Drug Data Report (MDDR; http://www.mdli.com/products/mddr.html), and **(d)** the Comprehensive Medicinal Chemistry (CMC; http://www.mdli.com/products/cmc/html) database. Dots are colored by the number of compounds found in each area, with red representing the highest density followed by yellow, green, blue and purple.

[48] when describing the benzodiazepine class of compounds. These types of structures can serve as ligands for a diverse range of receptors through variation of chemical substituents [49]. An example of this is shown in Figure 3, where hits from 127 combinatorial libraries were characterized against the observed activity at 15 GPCRs. Several of the libraries are active, but a prominent group of libraries possessing a common core structure has >25% of the total activity observed for the GPCRs panel. Analysis based on the type of cognate ligand for the GPCRs reveals little preference for either small-molecule- or peptidic- (≥30 amino acids) ligands. Therefore, the actives from these libraries contain structures with an affinity that is independent of the natural ligand for the target. Further screening of this set of libraries (Fig. 2; highlighted in blue) against targets other than GPCRs has shown that they are capable of yielding hits with targets such as integrins and kinases. This information can be used further to move from structurally diverse libraries to orientated libraries aimed at various molecular target classes. Structural information derived from both preference and privileged libraries is currently being used to make libraries with improved biological activity.

Different strategies are required for screening collections of millions of compounds. Table 1 gives statistics from a recent screening campaign of a nuclear receptor screened using a homogenous format in 1536-well plates. From the figures given in Table 1, it is clear that large combinatorial library screening involves several steps. Multiple compounds are arrayed per well and then eluted from the solid support. Pooling large chemical libraries is common practice in the pharmaceutical industry as it provides significant savings in time and reagent cost [31,50]. Generally, one copy of the library is arrayed at ~10 compounds per well

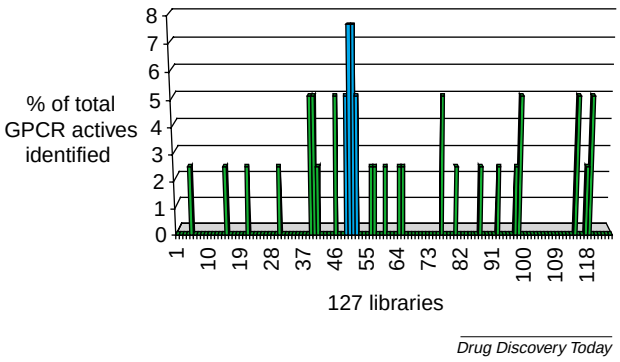

Figure 3. Activity profile for 127 libraries paneled against 15 G-protein-coupled receptors (GPCRs). Libraries highlighted in blue have a common core structure.

and then subjected to a biological screen. In the screen described here, the hit rate (defined as <50% of the positive control) was 0.26%. The strategy used to follow-up these 879 hits takes advantage of the library construction and arraying process. As each library is synthesized around a common core structure, and each library is further divided into sub-libraries containing a common substituent, two hits identified within a sub-library are analogs of each other. This has important implications in the analysis of combinatorial libraries arrayed in this way. Unlike screening historical compound libraries, where the number of analogs is often limited, a combinatorial library contains many thousands of analogs. The purpose of the primary screen is therefore to identify an active chemical series rather than to identify all active structures in the collection. This can be achieved by focusing on those sub-libraries that exhibit the most promising activity. In the example given in Table 1, the 879 hits were found among five libraries. Those sub-libraries showing the highest potency and frequency of hits were screened using a single-compound-per-well technique at threefold redundancy. (Arraying library compounds yields a random population and so three equivalents must be screened to achieve 95% coverage of all the compounds within a sub-library; see Box 1.) This led to a total of 178 decoded structures that showed >50% inhibition of activity (compared to the positive control). Owing to the statistical nature of the screen, many of the decoded structures will be replications of each other (duplicates, triplicates and quadruplicates are often observed) [51]. Therefore, the number of unique structures further reduces this set of structures to 87. In this particular example, 18 of the structures were re-synthesized and quantitative IC_{50} values were obtained. This analysis confirmed activity in the five libraries, yielding five active confirmed chemical series for the target. The best compounds had potencies in the 20–100 nM range. However, two of these series had chemical structures with poor physicochemical characteristics and were therefore not advanced to the hit-to-lead optimization stage. As outlined later, these screening data provide significant information on structure–activity relationships (SARs), which can subsequently be used to advance the series into viable lead compounds.

Table 1. Examples of screening statistics

Parameter	Number
Compounds screened	3.87×10^6
Wells screened	331,776
Hits at <50% of control	879
Compounds decoded	178
Unique structures	87
Compounds re-synthesized	18
Active series confirmed	5
Series entering hit-to-lead	3

Box 1. Screening statistics in combinatorial libraries

During the synthesis of encoded combinatorial libraries, compounds are built on beads with each bead containing a chemical tag that indexes the synthetic history of that particular compound. Further complexity is achieved by repeating the process of treating batches of beads with different reagents, combining the batches and then dividing the batches equally so that the next set of reagents can be added. The beads are arrayed, the compounds are eluted off the beads, and the eluant is assayed while the bead is kept separately so that the structure of hits can subsequently be decoded. Each bead contains one compound. The number of beads that must be arrayed to ensure that all possible compounds present in the library are assayed is given by the following formula:

$$(1 - e^{-L}) \times 100 = \%\ \text{of the library to be assayed at least once}$$

Where L = the number of library equivalents. One equivalent is defined as the number of beads equal to the number of distinct library members.

For example, a combinatorial library built-up in three steps by combining 31 substituents in the first step, 31 substituents in the second step and 9 substituents in the last step contains $31 \times 31 \times 9 = 8649$ distinct library members. Therefore, if one equivalent of this library is arrayed (8649 beads), the fraction of the library assayed at least once is 63%, whereas if three equivalents were arrayed the coverage would increase to 95%.

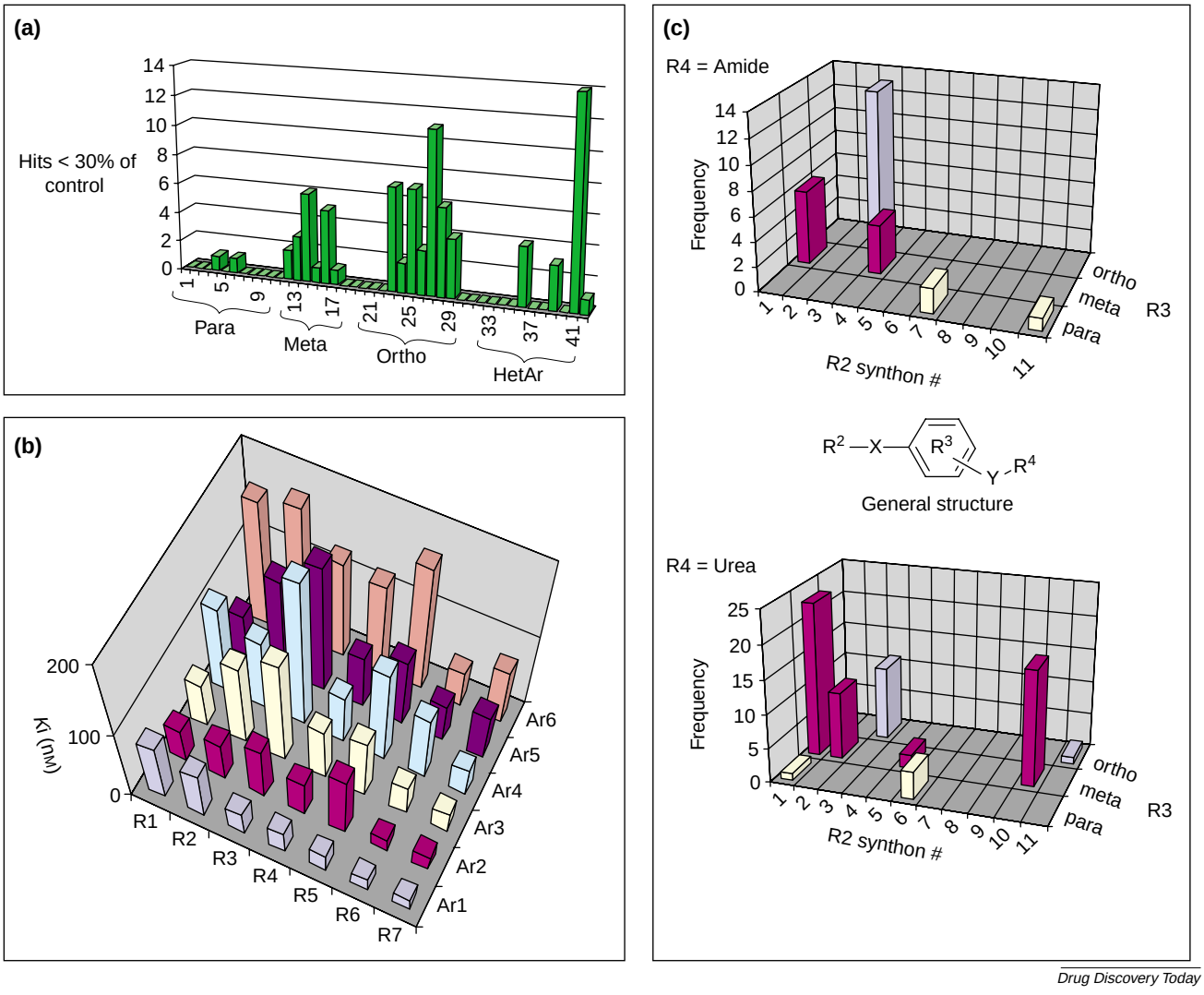

Figure 4. Structure–activity relationships from the combinatorial libraries. **(a)** Distribution of activity versus sublibrary for an 8500 member library divided into 42 sublibraries. The sublibraries 1–29 have an aryl piece with either an ortho (sublibraries 1–10), meta (sublibraries 11–20) or para (sublibraries 21–29) substituent, while sublibraries 30–42 are defined by a heteroaryl (HetAr) piece. **(b)** Modification of two variables during a hit optimization effort. **(c)** Combinatorial effects in a lead optimization library.

Analysis of combinatorial screening data

The use of combinatorial strategies to obtain information on SARs originates from genetic studies on various biological systems. In these cases, an oligonucleotide cassette containing the appropriate combinations of nucleotides is placed in the gene of interest. The system is then placed under a selective pressure (such as temperature or complementation strategies) to reveal a set of preferred amino acids at different positions of the protein product. Similarly, during a typical screening campaign, chemical combinatorial libraries are applied to biological targets and then assayed to reveal molecules that are selected by the biological target. As described previously, combinatorial libraries are based around a common core and can then be divided into sub-libraries that have a common substituent. Once activity is identified in a particular library, sub-library information can be used as illustrated in Figure 4a. In this example, potent activity was observed in the library and the frequency of hits obtained shows a clear preference for ortho-substituted aryl pieces and heteroaryl pieces at position three. This information was used to make choices about which chemotypes should be placed at this position during further optimization. The synthetic quality of the combinatorial library must be taken into consideration when interpreting such primary screening data. At Pharmacopeia, a library quality-assurance protocol has been developed [52]. This is a statistically based method that combines single-bead LC–MS analysis with tag-decoding

to confirm the presence of putative library compounds. These data taken together with quality-control compounds used to develop the library synthesis protocol are crucial when interpreting whether the presence or absence of a substituent is owing to its biological activity alone. In this way, the screening protocol outlined previously identifies active structures that can be cross-referenced with a particular affinity profile to reveal the interdependent relationships of structural variations.

Herein lies the real distinction of combinatorial chemistry: it provides a multivariable dataset that can be rapidly examined during screening and used to guide subsequent hit-to-lead optimization steps. Experiments that vary only one parameter at a time will often fail to reveal complex interactions between factors and will miss useful combinations [10,53,54]. Examples of how this information can be used in the downstream optimization of hits are outlined in the following section.

Hit-to-lead optimization: combinatorial effects

When active structures are identified from a screening campaign, the next step is often to synthesize a set of compounds containing modifications in two or more domains, with the key objective of establishing initial SARs of the hits. Any positive or negative movement in potency and/or selectivity will be useful in determining whether the properties are tractable. For example, a small set of compounds (Fig. 4b) with modifications in two substituents was prepared based on a 50 nM hit from an encoded-library screen against a nuclear receptor target. The Ar1–Ar7 groups were taken from the active structures identified from the encoded library, and the R1–R7 groups were novel substituents that had not been included in the original library. A SAR trend is observed for both the Ar and R dimensions. In addition, improved potency is achieved for the preferred combinations. Thus, combining Ar1 or Ar2 with R6 or R7 yields compounds with a binding potency of <20 nM. In this example, the choice of substituents was based on analysis of the screening data. These data suggest that improving the potency of the initial hit is highly tractable.

Possible combinatorial effects can also be examined, that is, how the change in one library substituent modifies the effect on activity of other substituents. Using an approach where multi-variables are examined early on in a program has the advantage of providing a broad survey that explores all major avenues. A representative example of a combinatorial effect on activity is illustrated in Fig. 4c. An encoded lead-optimization library with four combinatorial steps was screened against a GPCR target. The frequency of substituents present in active structures that met the potency criterion were plotted with respect to two variables, the R2 substituents and the R3 *para-, meta-* or *ortho*-positional isomers. When R4 is an amide, R2 substituents 7 and 11 are preferred for the R3 *para*-isomer. No structure that met the potency criterion contains substituents 1 and 4 in R2 when the R3 group is *para*; however, these two substituents are the most active groups when the R3 is a *meta*-isomer. Moreover, substituent 3 in R2 is preferred for the *ortho*-isomer but not in the two other positional isomers. Similar combinatorial effects were observed for compounds with a urea R4 group (see Fig. 4c).

Concluding remarks

The pressure to maximize the success of combinatorial chemistry and screening technologies is now increasing as many of the top pharmaceutical products are scheduled to go off-patent within the next four years. The human genome project has provided ~500–1000 new human genes with relevance to drug discovery, which should yield from 1500 to 10,000 therapeutically relevant intervention points [2]. The future of combinatorial chemistry must expand not only in the classical drug discovery paradigm but also to chemical genomic drug discovery, where libraries are used to validate and define novel genomic targets [55]. Expansion of functional cell-based assay technologies will be paramount to providing the information needed to define these novel biological interactions [56]. Focused libraries based on internal discovery efforts need to be streamlined to leverage both activity and drug-like properties [57] to reduce the high rate of clinical failure.

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References

1 Ray, L.B. and Gough, N.R. (2002) Orienteering strategies for a signaling maze. *Science* 296, 1632–1633
2 Workman, P. (2001) A gold mine for therapeutic targets. In *Farnesyltransferase Inhibitors in Cancer Therapy: Signal Transduction Pathways* (Sebti, S.M. and Hamilton, A.D., eds), pp. 1–20, Humana Press
3 Drews, J. (2000) Drug discovery: a historical perspective. *Science* 287, 1960–1964
4 Stadel, J.M. *et al.* (1980) A high affinity agonist β-adrenergic receptor complex is an intermediate for catecholamine stimulation of adenylate cyclase in turkey and frog erythrocyte membranes. *J. Biol. Chem.* 255, 1436–1441
5 Samama, P. *et al.* (1993) A mutation-induced activated state of the β2-adrenergic receptor. Extending the ternary complex model. *J. Biol. Chem.* 268, 4625–4636
6 Schwyzer, R. (1980) Organization and transduction of peptide information. *Trends Pharmacol. Sci.* 1, 327–331
7 MacDonald, R.L. and Olsen, R.W. (1994) GABAA receptor channels. *Annu. Rev. Neurosci.* 17, 569–602
8 Thorpe, D.S. (2000) Forecasting roles of combinatorial chemistry in the age of genomically derived drug discovery targets. *Comb. Chem. High Throughput Screen.* 3, 421–436

9 Fauchere, J.L. (1999) Discovery and design of peptide and non-peptide ligands to G protein-coupled receptors. *NATO Sci. Ser. Ser. A.* 307, 52–63
10 Appell, K. *et al.* (2001) Combinatorial chemistry and high-throughput screening in drug discovery and development. In *Handbook of Modern Pharmaceutical Analysis*, pp. 23-56, Academic Press
11 Lange, G. *et al.* (2002) Principles governing the binding of a class of non-peptidic inhibitors domain of src studied by X-ray analysis. *J. Med. Chem.* 45, 2915–2922
12 Al-Obeidi, F.A. and Lam, K.S. (2000) Development of inhibitors for protein tyrosine kinases. *Oncogene* 19, 5690–5701
13 Dalgarno, D.C. *et al.* (2000) Signal transduction drug discovery: targets, mechanisms and structure-based design. *Curr. Opin. Drug Discov. Devel.* 3, 549–564
14 Spencer, R.W. (1998) High-throughput screening of historic collections: observations on file size, biological targets, and file diversity. *Biotechnol. Bioeng.* 61, 61–67
15 Sawyers, C.L. (2002) Rational therapeutic intervention in cancer: kinases as drug targets. *Curr. Opin. Genet. Dev.* 12, 111–115
16 Shawver, L.K. *et al.* (2002) Smart drugs: tyrosine kinase inhibitors in cancer therapy. *Cancer Cell* 1, 117–123
17 Hooft van Huijsduijnen, R. *et al.* (2002) Selecting protein tyrosine phosphatases as drug targets. *Drug Discov. Today* 7, 1013–1019
18 Schindler, T. *et al.* (2000) Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science* 289, 1938–1942
19 Adams, J. and Lee, D. (1999) Recent progress towards the identification of selective inhibitors of serine/threonine protein kinase. *Curr. Opin. Drug Discov. Devel.* 2, 96–109
20 Dumas, J. *et al.* (2000) Discovery of a new class of p38 kinase inhibitors. *Bioorg. Med. Chem. Lett.* 10, 2047–2050
21 Sarmiento, M. *et al.* (2000) Structure-based discovery of small molecule inhibitors targeted to protein tyrosine phosphatase 1B. *J. Med. Chem.* 43, 146–155
22 Xue, L. *et al.* (2001) A dual fingerprint-based metric for the design of focused compound libraries and analogs. *J. Mol. Model.* 7, 125–131
23 Ghosh, S. *et al.* (2001) Rational design of potent and selective EGFR tyrosine kinase inhibitors as anticancer agents. *Curr. Cancer Drug Targets* 1, 129–140
24 Dolle, R.E. (2001) Comprehensive survey of combinatorial library synthesis: 2000. *J. Comb. Chem.* 3, 477–517
25 Borchardt, A. and Still, W.C. (1994) Synthetic receptor binding elucidated with an encoded combinatorial library. *J. Am. Chem. Soc.* 116, 373–374
26 Baldwin, J.J. (1996) Design, synthesis and use of binary encoded synthetic chemical libraries. *Mol. Divers.* 2, 81–88
27 Chabala, J.C. (1995) Solid-phase combinatorial chemistry and novel tagging methods for identifying leads. *Curr. Opin. Biotechnol.* 6, 632–639
28 Burbaum, J.J. *et al.* (1995) A paradigm for drug discovery employing encoded combinatorial libraries. *Proc. Natl. Acad. Sci. U. S. A.* 92, 6027–6031
29 McMillan, K. *et al.* (2000) Allosteric inhibitors of inducible nitric oxide synthase dimerization discovered via combinatorial chemistry. *Proc. Natl. Acad. Sci. U. S. A.* 97, 1506–1511
30 Blasko, E. *et al.* (2002) Mechanistic studies with potent and selective inducible nitric-oxide synthase dimerization inhibitors. *J. Biol. Chem.* 277, 295–302
31 Myers, P.L. (1997) Will combinatorial chemistry deliver real medicines? *Curr. Opin. Biotechnol.* 8, 701–707
32 Sarshar, S. *et al.* (2000) 2,4,5-Trisubstituted imidazoles novel nontoxic modulators of P-glycoprotein mediated multidrug resistance. part 1. *Bioorg. Med. Chem. Lett.* 10, 2599–2601
33 Zhang, C. *et al.* (2000) 2,4,5-Trisubstituted imidazoles: novel nontoxic modulators of P-glycoprotein mediated multidrug resistance. Part 2. *Bioorg. Med. Chem. Lett.* 10, 2603–2605
34 Cohen, M.H. *et al.* (2002) Approval summary for imatinib mesylate capsules in the treatment of chronic myelogenous leukemia. *Clin. Cancer Res.* 8, 935–942
35 La Rosee, P. *et al.* (2002) Insights from pre-clinical studies for new combination treatment regimens with the Bcr-Abl kinase inhibitor imatinib mesylate (Gleevec/Glivec) in chronic myelogenous leukemia: a translational perspective. *Leukemia* 16, 1213–1219
36 Elices, M.J. ((2002**)**) BX-471(Berlex). *Curr. Opin. Invest. Drugs* 3, 865–869
37 Clark, D.E. and Pickett, S.D. (2000) Computational methods for the prediction of 'drug-likeness'. *Drug Discov. Today* 5, 49–57
38 Muegge, I. *et al.* (2001) Simple selection criteria for drug-like chemical matter. *J. Med. Chem.* 44, 1841–1846
39 Walters, W.P. *et al.* (1999) Recognizing molecules with drug-like properties. *Curr. Opin. Chem. Biol.* 3, 384–387
40 Yoshida, F. and Topliss, J.G. (2000) QSAR model for drug human oral bioavailability. *J. Med. Chem.* 43, 2575–2585
41 van de Waterbeemd, H. *et al.* (2001) Property-based design: optimization of drug absorption and pharmacokinetics. *J. Med. Chem.* 44, 1313–1333
42 Veber, D.F. *et al.* (2002) Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem.* 45, 2615–2623
43 Lipinski, C.A. *et al.* (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* 46, 3–26
44 Egan, W.J. *et al.* (2000) Prediction of drug absorption using multivariate statistics. *J. Med. Chem.* 43, 3867–3877
45 Ghose, A.K. *et al.* (1998) Prediction of hydrophobic (lipophilic) properties of small organic molecules using fragmental methods: An analysis of ALOGP and CLOGP methods. *J. Phys. Chem. A.* 102, 3762–3772
46 Teaque, S. *et al.* (1999) The design of leadlike combinatorial libraries. *Angew. Chem. Int. Ed. Engl. 38*, 3743-3748
47 Hann, M.M. *et al.* (2001) Molecular complexity and its impact on the probability of finding leads for drug discovery. *J. Chem. Inf. Comput. Sci.* 41, 856–864
48 Evans, B.E. *et al.* ((1988**)**) Methods for drug discovery: development of potent, selective, orally effective cholecystokinin antagonists. *J. Med. Chem.* 31, 2235–2246
49 Patchett, A.A. and Nargund, R.P. (2000) Privileged structures – an update. Ch. 26 *Ann. Report Med. Chem.* 35, 289–298
50 Landro, J.A. *et al.* (2000) HTS in the new millennium. The role of pharmacology and flexibility. *J. Pharm. Tox. Meth.* 44, 273–289
51 Appell, K.C. *et al.* (1996) Biological screening of a large combinatorial library. *J. Biomol. Screen.* 1, 27–31
52 Dolle, R.E. *et al.* (2000) A statistical-based approach to assessing the fidelity of combinatorial libraries encoded with electrophoric molecular tags. Development and application of tag decode-assisted single bead LC/MS analysis. *J. Comb. Chem.* 2, 716–731
53 Hunter, J.S. (1987) Applying statistics to solving chemical problems. *CHEMTECH* 17, 167–169
54 Topliss, J.G. (1972) Utilization of operational schemes for analog synthesis in drug design. *J. Med. Chem.* 15, 1006–1011
55 Croston, G.E. (2002) Functional cell-based uHTS in chemical genomic drug discovery. *Trends Biotechnol.* 20, 110–115
56 Inglese, J. (2002) Expanding the HTS paradigm. *Drug Discov. Today* 7, (Suppl.) S105–S106
57 Hobbs, D.W. and Guo, T. (2001) Library design concepts and implementation strategies. In *Combinatorial Library Design and Evaluation: Principles, Software Tools, and Applications in Drug Discovery* (Ghose, A.K. and Viswanadhan, V.N., eds) pp. 1–49, Marcel Dekker