

# Preface

The methods of drug discovery in the pharmaceutical industry have changed dramatically in the last two decades. By the late 1980s, a strong belief had emerged that drug development is purely a numbers game, with anticipated drop-out rates at each stage of the process such that only one compound out of 10,000 synthesized would survive to make it to the market. Technologies to accelerate both synthesis and screening were developed and adopted by virtually every pharmaceutical and biotech research division. The race for ever higher numbers had started and the implementation of high-throughput technologies resulted in quantum changes in both chemistry and *in vitro* biology that transformed the search for new drugs.

However, a counterwind began blowing in the late 1990s, when the number of New Drug Applications (NDAs) at the regulatory authorities dropped to its all time low, and it became evident that more compounds screened were not going to translate directly into more drugs discovered. High-throughput synthesis and screening has to be informed by knowledge of what has worked and not worked in the past, in short, medicinal chemical intuition. Paradigms such as Christopher Lipinski's "Rule-of-Five,"<sup>1</sup> resulting from an empirical analysis of the physical properties of successful drugs, represents a milestone in the codification of this intuition. Data mining strategies, assessments of diversity and "drugability," and the design of high-content biological screens have thus become indispensable in complementing high throughput chemistry and screening technologies. The melding of a number of disciplines into the field now known as chemical biology has created the foundation and driving principle for modern drug discovery. This volume encompasses the changes that have occurred in both chemistry and screening as applied to drug discovery. In addition, we have distinguished the conceptual from the operational advances in each area, although we recognize that, like a good tango duo, neither would progress without the other.

Perhaps nowhere is the interplay of conceptual and operational advances more apparent than in the organic chemistry of drug discovery, where high-throughput concepts and automation have had a truly transforming effect. Stimulated by the automation of peptide synthesis on solid support, the concepts now embodied in combinatorial chemistry have completely changed the way in which compound collections are assembled and structure-activity relationships (SARs) are explored. Reactions are accelerated by microwave and other non-traditional techniques, reaction workups are streamlined with solid-phase reagents, and parallel or mix-and-split formats enable chemists to make hundreds of analogues in the time they used to synthesize one or two. These changes have not only altered the operational aspects of organic synthesis, but also given rise to entirely new strategies in synthetic planning.<sup>2</sup> The design of a chemical library will depend on whether it is destined for a

discovery screen against a novel target, in which case diversity is key, or whether it will explore the SAR around a particular chemotype for a specific target. Will the library be based on a novel scaffold, or derived from a peptide or natural product? Should it be prepared by automated, parallel techniques, or can a biosynthetic pathway be pressed into service? Often there is no right answer to these questions, and debate is likely to continue for a long time on the key issue of “diversity:” how is it measured, how is it designed, and, ultimately, what does it mean in the context of a chemical collection?

High-throughput screening (HTS) has always played an important role in the lead-identification phase of drug discovery, so the impact from recent technological advances is more operational than conceptual. Nevertheless, HTS, and now ultra-HTS, has dramatically accelerated the discovery of initial hits from compound collections by increasing both the quantity and quality of information obtained. Increases in capacity have been driven by increases in the size of compound libraries, and modern screening technologies have greatly improved the analysis of the interaction of small molecules with novel pharmacological targets.

Assay methods are increasingly moving away from the use of radioisotopic labels. Modern fluorescent technologies now play an important role and are widely employed for the evaluation of more complex target systems, such as peptide–protein or protein–protein interactions. Reporter-gene assays have been developed to analyze functional responses to the activation of cellular signaling systems, such as membrane receptors, receptor tyrosine kinases, and ion channels. Using these techniques, inhibitors, allosteric, and transcriptional modulators, in addition to direct agonists, can be identified from chemical libraries. Moreover, both fluorescence- and reporter gene-based screening systems provide sufficiently high sensitivity that interactions of high-affinity ligands with targets present in low abundance can be probed.

At the other extreme, there is also interest in identifying low-affinity ligands for pharmacological targets. In fragment-based screening, the goal is to identify pharmacologically active partial structures. If different fragments for adjacent binding sites on the target are found, they can be linked to form ligands with higher affinity. As the small probe molecules normally bind with low affinity, this approach requires specific tools to detect and characterize their interaction with the biological target. NMR-based techniques have emerged as the most powerful of the methods used to detect low-affinity ligands in biological systems.

In addition to dynamic range and throughput, compound consumption remains one of the major issues in HTS. Miniaturization as well as high-density array formats have not only greatly improved the throughput of HTS campaigns, but also significantly reduced the amount of chemical material needed to determine ligand affinity. The volume of an individual assay well nowadays is in the low microliter range, down considerably from the milliliter volumes still used in the early 1980s, yet the quality of biological data obtained remains very high. Increases in daily screening throughput from automation and miniaturization have in turn required expanded capabilities in compound library storage and material handling. Technology continues to advance, with developments in nanotechnology and microfluidics opening new perspectives in reducing both time and sample amount. Chemical microarrays and bead-based applications may further enhance performance, although these formats have not yet been

broadly introduced in pharma-screening laboratories because new detection capabilities are required. However, as we look to the future, it is clear that these formats will find their place in the initial hit identification process as the techniques advance.

The newer screening techniques are able to analyze many different types of ligand–target interactions and have provided research teams with a huge number of hits from HTS campaigns and, in turn, leads for the optimization phase of drug discovery. However, the output of preclinical and clinical candidates has failed to keep pace. Inadequate pharmacokinetic properties or adverse toxicological effects have been identified as the predominant reasons why drug candidates fail during development.<sup>3,4</sup> The pharmaceutical industry has responded by paying increased attention in the early phases of library design and lead identification to important “drug-like” attributes, such as metabolic stability, physicochemical properties, and membrane permeability. In fact, early awareness and corrective action to avoid potential liabilities has resulted in a modest decrease in the number of compounds that fail because of adverse pharmacokinetic properties.<sup>5</sup> With the expansions of the knowledge base, *in silico* predictive methods have become available to assess very large compound datasets, helping scientists to classify and rank compound collections or library designs according to their predicted properties or compliance with established rules. The potential for compounds to interact with cytochrome P450 enzymes<sup>6</sup> or to exhibit favorable absorption characteristics<sup>7</sup> are only two of the many examples of these predictive methods. Such machine-learning techniques are likely to play an increasingly important role in pharmaceutical screening and optimization processes in the future.

The concept of understanding more about a compound’s biological behavior earlier in the process underlies the new concept of “high-content screening” (HCS). Indeed, it is in this area that the greatest conceptual advances in screening can be anticipated. The HCS approach has led to the development of a new generation of dedicated instruments that enable intracellular signaling pathways and cascades to be elucidated and analyzed. In large-scale, multiplexed, cell-based assays, the large amount of information that is generated can be captured and processed for this purpose. HCS combines specific components of assay and reagent design with robust instrumentation for automated fixed-end-point and live-cell kinetic analysis to generate information-rich data from multiple cellular targets.<sup>8</sup> By using multiple fluorescent reporter systems, combined with high-resolution imaging and high-throughput image processing, scientists can observe multiple intracellular events on a cellular level. HCS enables a functional analysis of how potential drug candidates modulate a particular pathway or target in living cells and has evolved into an integrated solution for accelerated drug discovery.

Over the past decade, drug discovery has undergone a dramatic evolution. It is clear that no discipline stands alone, that no breakthrough technology is “the solution,” indeed, that integration of techniques and knowledge at the earliest stage is essential. The title of this volume emphasizes diversity, and we think the content of the following chapters reflects this concept in many ways. The closer integration of chemistry, biology, and technology that is transforming drug discovery has stimulated advances in numerous areas at the interfaces of these disciplines. While the individual chapters take on separate aspects of this on-going transformation, each

chapter itself reflects an integration of different disciplines, techniques, or viewpoints. While the specific descriptions in the chapters that follow can only be snapshots of this evolving field, the concepts of cross-disciplinary integration, incorporation of medicinal chemical knowledge from the outset, and high-capacity information acquisition and analysis will always be relevant. It is our fervent hope that this volume will not only give readers a sense of the state of the art of drug discovery, but also stimulate the next generation of scientists to think how they could advance the field themselves.

## References

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