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Daniel A. Erlanson and Wolfgang Jahnke

Fragment-based Drug Discovery

Lessons and Outlook

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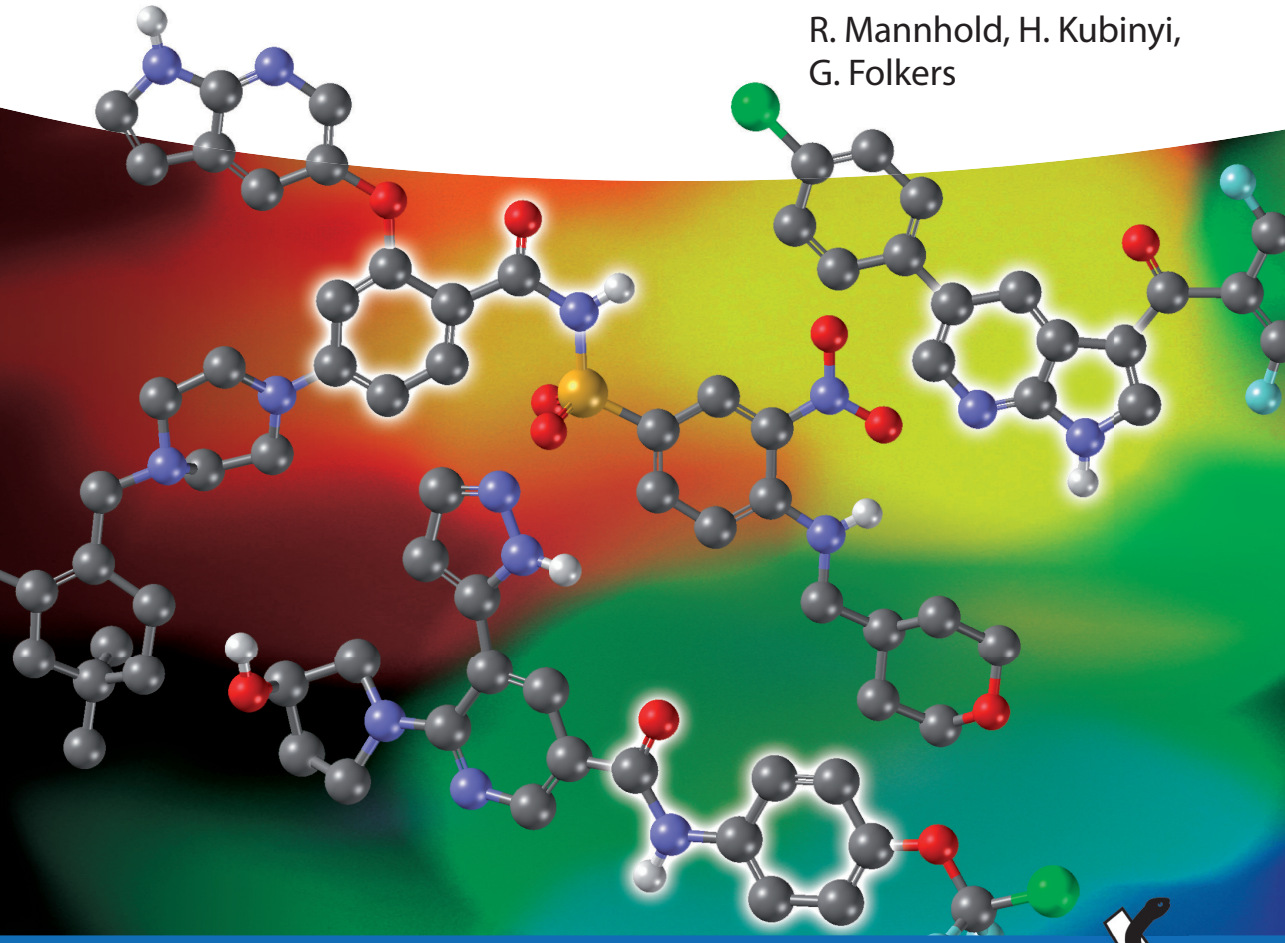

Methods and Principles in Medicinal Chemistry

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Preface

Just two decades ago, Stephen Fesik initiated fragment-based ligand design by developing an NMR-based method to search for small, low-affinity ligands in adjacent binding pockets of a protein and to link them to a high-affinity ligand [1]. A broader use of this approach was hindered both by its limitation to relatively small proteins and by a patent application. However, within short time alternative methods emerged, originally based on different NMR techniques, later using protein crystallography. Thus, structure-based design was not any longer restricted to "large" molecules: libraries of much smaller fragment-type compounds were tested experimentally or screened in silico, with the advantage that a small ligand has a much better chance to fit a certain binding site. In further steps, the ligand can grow into the environment of its pocket or can be linked to an adjacent fragment. The only critical step in fragment combination is the search for a linker that combines the fragments in a relaxed, bioactive conformation, optimally stabilizing this favorable conformation.

Ten years later, in 2006, time was already ripe to review the techniques and the accumulated experience in fragment-based ligand design: Wolfgang Jahnke and Daniel Erlanson edited the very first book on this topic [2]. Now, another 10 years later, the discipline has significantly developed and a major number of drug candidates resulted from its use. Thus, we are very grateful that both experts agreed to edit not only a new edition but also a completely new book on fragment-based design. In its introductory section, leading scientists of this area review the role of fragment-based approaches in lead finding and the selection of appropriate targets. Next, an overview on chemical space is provided. The second section discusses library design and various screening techniques, together with a major number of issues that are relevant in fragment-based ligand discovery. The last section presents a significant number of success stories, providing evidence for the broad applicability of fragment-based design in drug research.

As last time, we are very grateful to the editors Daniel Erlanson and Wolfgang Jahnke for assembling such a unique collection of important topics, as well as to all chapter authors for their excellent work. Last but not least we thank the

publisher Wiley-VCH, in particular Waltraud Wüst and Frank Weinreich, for their valuable contributions to this project and the entire series.

Düsseldorf — *Raimund Mannhold*
Weisenheim am Sand — *Hugo Kubinyi*
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October 2015

Reference

1 Shuker, S.B., Hajduk, P.J., Meadows, R.P. and Fesik, S.W. (1996) Discovering high-affinity ligands for proteins: SAR by NMR. *Science*, **274**, 1531–1534.

2 Jahnke, W. and Erlanson, D. eds., (2006) *Fragment-based Approaches in Drug Discovery (Volume 34 of Methods and Principles in Medicinal Chemistry*, eds.), R. Mannhold, H. Kubinyi, and G. Folkers Wiley-VCH Verlag GmbH, Weinheim.

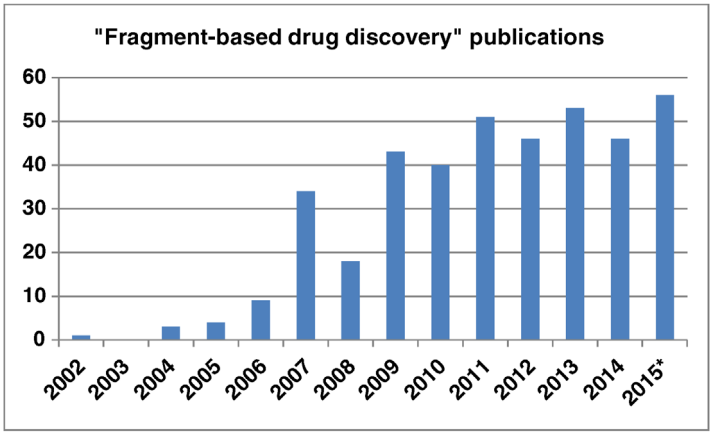
A Personal Foreword

> For the great things are not done by impulse, but by a series of small things brought together.
>
> *Vincent Van Gogh, 1888*

When Wiley-VCH asked us whether we would be willing to edit a new book on fragment-based drug discovery, our first reaction was panic. Editing a book is a daunting task, and having done it once already we knew well what was in store.

Our second reaction was to ask whether a new book was really needed. Since the very first book on fragment-based drug discovery was published by Wiley-VCH in 2006, six more books have appeared, along with dedicated journal issues and dozens of reviews. Was there anything new to say?

Happily, as you will soon discover, the answer is an emphatic yes! This is clearly illustrated by a search for publications containing the phrase "fragment-based drug discovery" in SciFinder®, as seen in the figure.



(*2015 numbers through August 25 only)

The past few years have seen a bumper crop of papers on the topic, and given that this search was run in August of 2015 this trend looks set to continue if not

accelerate. From its origins as a niche technique, fragment-based approaches have spread throughout the world to organizations large and small and are embraced by biologists, biophysicists, chemists, modelers, and more. More than 30 drugs derived from fragments have entered the clinic (http://practicalfragments.blogspot.com/2015/01/fragments-in-clinic-2015-edition.html), and one (vemurafenib) has already been approved. This book is a comprehensive view of where the field stands – and where it is going.

We would like to thank Wiley-VCH, especially Frank Weinreich and Waltraud Wüst, for encouraging us to undertake this project and patiently working with us through the inevitable but nonetheless frustrating difficulties and delays. We would also like to thank our contributors, all of whom are extraordinarily busy and accomplished scientists. We are thrilled with the response we received to our invitations and with the depth and quality of the chapters. Finally, we would like to thank you for reading. We hope that you will find something useful to apply to your own research: each of our fragmentary efforts advances the great human enterprise of drug discovery.

San Francisco — *Daniel A. Erlanson*
Basel — *Wolfgang Jahnke*
August 2015

Part I
The Concept of Fragment-based Drug Discovery

1
The Role of Fragment-based Discovery in Lead Finding

Roderick E. Hubbard

1.1
Introduction

Fragment-based lead discovery (FBLD) is now firmly established as a mature collection of methods and approaches for the discovery of small molecules that bind to protein or nucleic acid targets. The approach is being successfully applied in the search for new drugs, with many compounds now in clinical trials (see summary in [1]) and with the first fragment-derived compound now treating patients [2]. The approach has also had a number of other impacts such as providing starting points for lead discovery for challenging, unconventional targets such as protein–protein interactions [3–5], increasing the use of biophysics to characterize compound binding and properties, and providing small groups, particularly in academia, with access to the tools to identify chemical probes of biological systems [6,7].

The other chapters in this book will discuss the details and new advances in the methods and provide examples of how fragments have been used in specific projects. In this chapter, I will draw on my own experiences and view of the literature to discuss three main areas. First, I will review current practice in FBLD, highlighting how and when fragments have an impact on the drug discovery process. Second, I will then review how the ideas have developed, with particular emphasis on the past 10 years. I will discuss how fragment methods and thinking have been extended and refined and how these developments have affected the lead discovery process in drug discovery. Finally, I will discuss some of the areas where we can see that improvements in fragment methods could have further impact on discovery.

The discussion will focus on fragment-based discovery against protein targets. Although there are a few examples of fragments being used against RNA [8–10] and DNA [11] targets, the majority of reported campaigns are against proteins. Two types of protein target will be considered. The first shall be called conventional targets. These are proteins such as kinases where although it is never straightforward to achieve the required selectivity and balance of physicochemical properties in the compound, the proteins usually behave in most of the

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experiments and assays. Crystal structures are usually readily obtained, large amounts of pure, homogeneous, and functional proteins can be generated for biophysical studies, and the activity assays are robust and well understood. The second class of target shall be called unconventional targets. There are two types here – the first are protein–protein interaction targets such as the proapoptotic Bcl-2 family or Ras, where experience over the years has eventually established reasonably robust assays and although crystal structures take some time to determine and the protein does not always behave in biophysical assays, it is possible to establish structure-based discovery. The main challenge here is the nature of the binding sites, with often large, hydrophobic, and sometimes flexible sites. The second type of unconventional targets are the results of recent advances in our understanding of mammalian disease biology and consist of new classes of enzymes (such as the ubiquitin processing machinery [12]), disrupting multiprotein complexes, and proteins that are intrinsically disordered in some way (such as the one described in [13]). Here, the primary challenges are often in producing sufficient, homogeneous, functional protein for study, knowing what the post-translational modification state or even which complex is the true target and establishing robust assays to report on activity or binding. This last issue is often not appreciated – it can take a long time to establish the assays on new classes of target, not only because there is intrinsic variability in the behavior of the system but also because there is often not a tool compound available with which to validate the assay.

1.2 What is FBLD?

There are two distinctive features of fragment-based discovery compared to other approaches to lead finding. The first is that the discovery process begins with screening a small (usually 1–2000 member) library of low molecular weight (typically less than 20 heavy atom) compounds for binding to a particular site on the target. Key is the molecular weight of the fragments – they are big enough to probe interactions in the protein but small enough to minimize chances of unfavorable interactions. The second distinctive feature lies in the approach to optimizing these hits to lead compounds, either through careful, usually structure-guided, growth of the fragment or through merging information from fragments and elsewhere to generate optimized hits.

In many ways, fragments can be viewed as a state of mind – an approach to use the fragments as chemical tools to dissect what the requirements are for the chemical matter that affects a particular target in the desired way (affinity, selectivity) and using a combination of rational, usually structure-guided, and often biophysics-based methods for generation of the optimized compounds. We can define a fragment approach as one of intent – and that intent affects the strategy, methods, and thinking that is applied during the early parts of a discovery project. Detection and characterization of such weakly binding compounds can be

problematic for some classes of target, with concerns over false positive and false negative hits, changes in binding mode, and so on. So, fragment methods engender a questioning, problem-solving approach to research. This is carried through into the usually structure-guided evolution of the initial fragment hits, which allows careful assembly of compounds that bind with high efficiency combined with suitable compound properties.

1.3 FBLD: Current Practice

Figure 1.1 and its legend summarize the contemporary approach to fragment-based discovery followed by most practitioners. There are five main components to a fragment platform: a fragment library, a method for finding which fragments bind, characterizing how the fragments bind by determining structure and biophysical measurements, exploring fragment SAR to identify the best fragment(s) to progress, and using the fragment(s) to generate lead compounds. Figure 1.1 also emphasizes how information about binding motifs is combined with information from HTS hits, literature compounds, or virtual screening hits. Other chapters in this book will provide detail on each of these different areas. In this chapter, I am focusing on the impact fragments have had on the lead discovery process. This is best done with some examples.

1.3.1 Using Fragments: Conventional Targets

Conventional targets are ones with well-defined active sites (such as most enzymes) where structural information is readily available. It is usual to get a large number of fragment hits for such targets – at Vernalis our experience has been 50–150 validated hits from screening a library of about 1500 fragments [14,15]. A lower hit rate can indicate there may be issues with progressing compounds against the target as discussed later. Modeling of the binding of these fragments can be helpful, but the most effective fragment to hit to lead optimization campaigns uses the detailed information available from experimental structures determined by X-ray crystallography (preferred) or if necessary by NMR. The main issue with NMR is the time it takes to generate structures. A suitable crystal form can generate many hundreds of crystal structures during the early months of a project, whereas it takes at best a few days for NMR methods to generate models for binding. In addition, NMR models rarely have the resolution to give confidence in some of the subtleties of binding mode necessary for design of selective compounds (such as for kinases).

The three main ways of using fragments are growing, merging, and linking. Figure 1.2a–c shows some representative examples that we can use to describe the essential features of each approach.

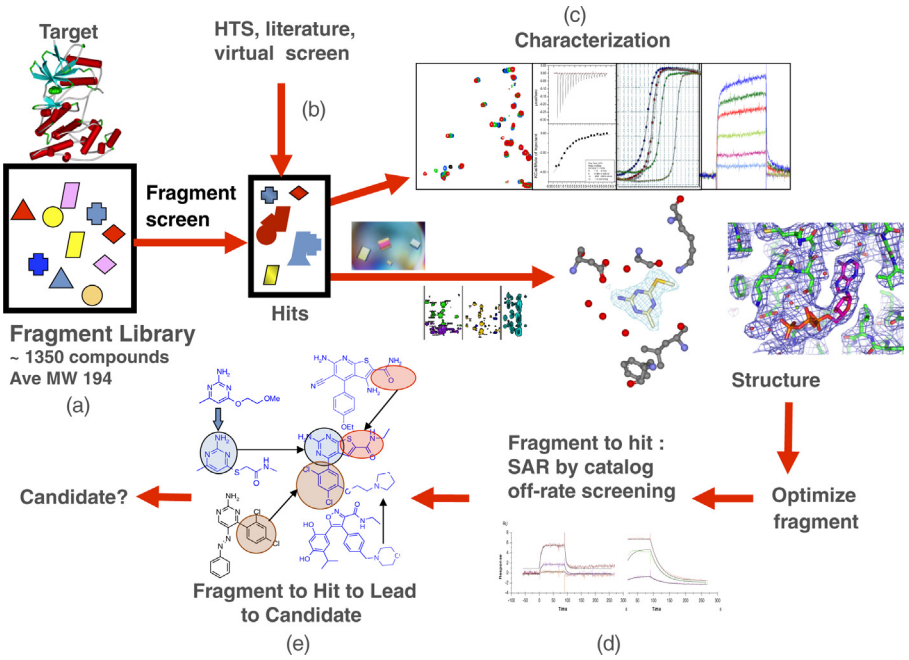

Figure 1.1 the FBLD process. There are five main components to a fragment platform. (a) Fragment library: there is an extensive literature on the design of fragment libraries [26,31,32,41]. The choice of compounds is constrained both by the demands of the screening methods (solubility, detection) and by the need to evolve the compounds (elaboration vectors, synthetic tractability) as well as avoiding reactive or toxic substructures. Key is the number of heavy atoms in the compounds. Analyses by Reymond [38,39] suggest that the number of possible lead-like compounds (chemical space) increases by around eightfold for each heavy atom. There are many approximations but this means that a fragment library of 1000 compounds of average MW 190 is equivalent to 10^8 compounds of MW 280 and 10^{18} compounds of MW 450. (b) Fragment screening: Table 1.1 summarizes the experiences at Vernalis over the years that are variously described elsewhere [15]. For all techniques, the main limitations are whether the protein target can be prepared in a suitable format for screening and whether the fragments are sufficiently soluble. The most robust method of screening is ligand-observed NMR, which has the dynamic range (typically from 5 mM to 100 nM) seen for fragment binding and particularly important for unconventional targets, as the integrity of the ligand and protein is checked at each experiment. (c) Characterizing fragment binding: for conventional targets, it is often possible to rapidly determine crystal structures of the fragment binding to the protein and, if the biochemical or binding assay is not suitable, use a biophysical method to validate and if possible quantify potency. For unconventional targets, this step is particularly important as the targets can have challenging binding sites, where conformational flexibility or large hydrophobic surfaces can challenge reliable detection of fragment binding. NMR methods can be used for unconventional targets, ranging from binding site localization (HSQC) to NMR-guided models (measuring NOE distances from ligand atoms to protein residues) and full structure determination. These are constrained by the size of the protein and requirement for isotope labeling. (d) Fragment SAR and optimization: there are two well-established methods – (1) SAR by catalog where features of the fragment are used to identify commercially available compounds for purchase and assay and

Table 1.1 A summary of the characteristics of the most widely used fragment screening methods.

Method	Sensitivity	Issues
Ligand-observed NMR – a number of NMR experiments (STD [82], Water-Logsy [83], and CPMG [84]) detect binding of a ligand to the protein	10 mM–100 nM	Requires large amounts of protein (many 10 s mgs) but the most robust method for detecting weak binding. Each experiment confirms that the ligand and protein maintain their integrity in solution; the use of a competitor ligand to displace the fragment can identify nonspecific binding. These features make the technique particularly suitable for weak binding to challenging targets. Requires careful design to identify allosteric or cryptic binding sites
Protein-observed NMR – HSQC experiment detects changes in the local environment of ^{15}N or ^{13}C nuclei as ligand added	5 mM–100 nM	Requires isotopic labeling of the protein; limited to proteins<35 kDa; can titrate ligand onto protein and determine K_D; pattern of changes in spectra can confirm the same binding site for different ligands and identify allosteric sites; assignment of spectrum allows localization of site
X-ray crystallography: either cocrystallization (crystals formed from the preformed protein–ligand complex) or soaking (high concentrations of ligand added to apo crystals)	All affinities	Cocrystallization can require different crystal conditions for each ligand. Soaking of apo crystals requires crystal form with accessible protein binding site; depending on crystal form can identify cryptic sites Crystal structure provides information-rich description of protein–ligand interactions ready for design
Surface plasmon resonance [47]; monitor molecular weight change as one component flows past the other attached to a surface	500 μM lower limit	Two modes – direct binding (protein attached, ligand flows) allows kinetics (k_{on} and k_{off}) to be measured; indirect, or affinity in solution, where tool compound attached and protein (in the presence of possible fragment) is flowed past. Main issue is immobilization and integrity of protein on surface

(*continued*)

(2) detailed design of bespoke compounds to optimize the fragment itself and explore potential vectors for elaboration. More recently, there have been new methods such as off-rate screening [16] that allow rapid profiling of compounds where substituents have been added to particular positions on the fragment, prospecting for suitable vectors for fragment evolution. This can be particularly important when limited structural information is available. (e) Fragment to candidate: medicinal chemistry optimization, supported where possible by rapid crystal structure determination, to bring together information from the portfolio of fragments, hits, HTS, literature, and so on to design and optimize lead compounds.

Table 1.1 (*Continued*)

Method	Sensitivity	Issues
Enzyme/binding assays	100 μM lower limit usually	The high concentrations of ligand interfere with most formats of assay preventing detection of mM binding fragments; effective for some assay formats and for well-defined active sites – for example, kinases
Isothermal titration calorimetry (ITC) [85]	1 mM–10 nM	Requires too much protein and ligand to be useful for screening, but the most robust method for measuring K_D as long as the interaction involves a change in ΔH
Mass spectrometry	100 μM	Requires protein/buffer system that "flies" in the mass spectrometer and an interaction that can survive in the gas phase. Effective for covalent interactions –too variable for weakly binding fragments
Weak-affinity chromatography [51] – immobilize the target on a silica column, then use LC–MS to identify retained ligands	1 μM upper limit	A cheap way of measuring weak interactions (using simple LC–MS equipment). As for SPR, the main limitation is attachment of protein to surface and behavior of the fragments on the surface
Thermal shift analysis (TSA) [86] – measure the melting temperature of the target by monitoring the increase in fluorescence as the target is heated up in the presence of a dye plus and minus the ligand	500 μM lower limit	A relatively reliable technique for detecting binding of ligands that bind better than 10 μM, but many false positives and negatives in detecting fragment binding – the change in melting temperature is too small to measure. Uses small amounts of material and inexpensive instrumentation

Fragment linking is a conceptually very attractive idea – find two fragments that bind in adjacent sites and achieve a high-affinity compound by linking them together.This was the basis of the initial SAR by NMR approach, but with a few exceptions, only the Abbott group (such as summarized in Figure 1.2a, see also Table 1.3), and the follow-on work by Fesik at Vanderbilt (see later), has

Figure 1.2 (a) Evolving fragments – linking. The SAR by NMR approach was developed by the Abbott group in the 1990s [22] (see also the reviews [63,64]). Protein-observed NMR screening of a library identifies the first site binder (screen 1) that can then be optimized (optimize 1). The second screen (screen 2) is then performed in the presence of an excess of the optimized first site binder to identify the second site binder that can also be optimized (optimize 2). NMR structure determination identifies appropriate vectors for linking the two fragments (link) to give a compound that can then be optimized. The first disclosed example was for FKBP [22]; the first drug discovery project was on stromelysin [65] and arguably the most successful was for the Bcl-2 family of proteins [66–69]. For stromelysin, compound **1** was not from screening but is a known metalloprotease binding motif. Screening in the presence of **1** identified compounds such as **2** that after

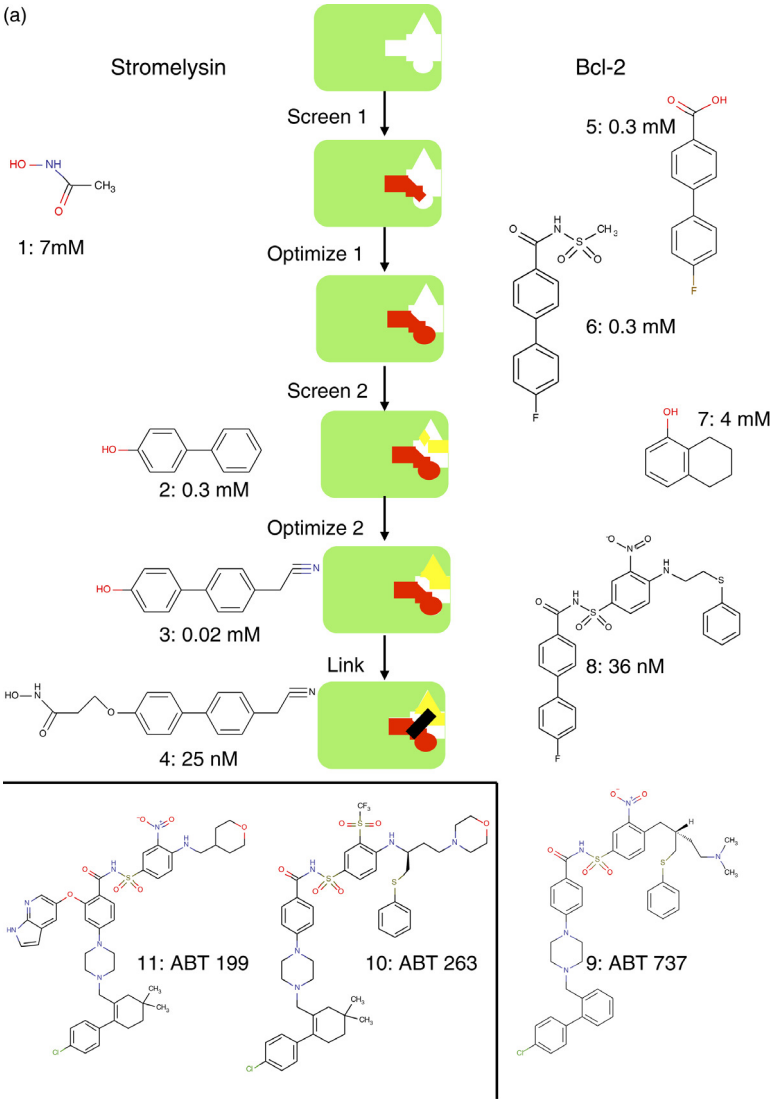

Figure 1.2 (a) (***Continued***) optimization gave **3**. Combining these in **4** very neatly demonstrates the power of the method – a large increase in potency, clearly retaining the two weakly binding fragments. For the Bcl-2 family, the evolution from the two site binding fragments **5** and **6** is less obvious in compound **7**, although the method did provide starting points for chemistry where conventional HTS failed. A considerable amount of medicinal chemistry optimization was needed to generate ABT-737 [66] that briefly entered clinical trials, followed by ABT-263 [70] with better drug-like properties though still with a dual Bcl-2/Bcl-x_L profile that can give undesired pharmacology. This has recently been succeeded in the clinic by the more Bcl-2 selective ABT-199 [71]. With few exceptions [72], the continued champion of the linking approach is Fesik, now at Vanderbilt (see Figure 1.2d). Most other practitioners find it difficult to identify such multiple sites and commit such dedicated chemistry resources to a linking strategy.