Recent Developments in Fragment-Based Drug Discovery

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Perspective

Recent Developments in Fragment-Based Drug Discovery

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1. Introduction

The field of fragment-based drug discovery (FBDD[a]) has developed significantly over the past 10 years and is now recognized as a tangible alternative to more traditional methods of hit identification, such as high throughput screening (HTS). The number of commercial and academic groups actively engaged in fragment-based research has increased, and as a consequence, there has been continued development and refinement of techniques and methods. From its inception, the fragment-based approach had two central tenets that were critical to its success and that have set it apart from HTS and other hit identification techniques. The first is the concept that chemical space can be more efficiently probed by screening collections of small fragments rather than libraries of larger molecules. The number of potential fragments with up to 12 heavy atoms (not including three- and four-membered ring structures) has been estimated at 10^7,[1] whereas the number of potential druglike molecules with up to 30 heavy atoms is estimated at more than 10^{60}.[2] Therefore, a much greater proportion of "fragment-like" chemical space can feasibly be screened in FBDD compared to "druglike" chemical space covered in a HTS where molecular size is much larger. The second idea is that, because by definition fragment molecules are small in size (typically less than 250 Da), they should typically bind with lower affinity to their target protein (micromolar to millimolar range) compared with druglike molecules that can form many more interactions (nanomolar to micromolar range) but that the binding efficiency per atom is at least as high as for larger hit molecules. Implicitly, the screening techniques employed in FBDD must be correspondingly much more sensitive than a HTS bioassay. Generally, sensitive biophysical techniques are employed to detect these weak binding events and to characterize the fragment interactions with the target active site. Nuclear magnetic resonance (NMR) and protein X-ray crystallography have been used extensively in fragment-based research because these techniques are highly sensitive in detecting low affinity fragment binding and also give information about the fragment−protein interactions being formed.

There have been a number of recently published general review articles that have discussed the various aspects of the FBDD field.[3–21] In addition, there are now two books on the subject.[22,23] In this journal in 2004, Erlanson et al. summarized the major developments in FBDD since the original publication by Fesik and co-workers of the "SAR by NMR" approach in the late 1990s.[3,24] Particular note was given to the biophysical methods employed to screen for fragment binding and the merits and drawbacks of each of these techniques, along with the approaches that can be used to optimize fragments into lead molecules. Herein, the trends and developments over the past 4 years will be outlined and some selected examples that are illustrative of the approaches being utilized by those active in the field examined. Additionally, this review will look in some detail at representative protein−ligand complexes observed between fragment-sized molecules and their protein targets from the Protein Data Bank (PDB). Finally, some conclusions will be drawn from these data and the future of FBDD discussed.

2. Trends and Developments

In the first part of this review we will examine how things have evolved and developed in the field of FBDD over the past 4 years.

2.1. Fragment Screening. Fragment-based screening has an intuitive appeal. The success of pharmaceutical companies like Abbott and biotechnology companies such as Astex Therapeutics, SGX Pharmaceuticals, and Plexxikon in developing fragments into clinical candidates has influenced the chemistry community, prompting fragment screening efforts in many other industrial and academic institutions.[16]

In industry over the past 3−4 years, a great deal of effort has been given to establishing fragment-based screening, and it is now generally being implemented as a complementary strategy to HTS. This is in some part due to the fact that investments made during the 1990s in HTS and combinatorial chemistry have not yielded success for more challenging classes of drug targets. However, despite the obvious efforts to implement fragment screening, there are significant cultural and practical issues to overcome within large companies to apply this new methodology in an effective manner. In particular, after identification of fragment hits, optimization to a more conventional potency range will often be difficult without structural information. Significant up-front investment in structural biology is required both to establish the binding modes of fragments within the active site of target proteins and to eliminate any false positives. This commitment to timely structural biology may be difficult to achieve in practice in large organizations, particularly when only a proportion of targets are readily amenable to 3D-structure determination. Another issue is that fragment hits with low or undetectable potency in a biological assay may initially appear less attractive to medicinal chemists when compared with conventional HTS hits with higher potency.

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[a] Abbreviations: FBDD, fragment-based drug discovery; HTS, high-throughput screening; HCS, high-concentration screening; LE, ligand efficiency; HAC, heavy atom count; GE, group efficiency; SBDD, structure-based drug design; MWT, molecular weight; PDB, Protein Data Bank.

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In contrast, in an academic setting assembling a small library of fragments and screening using a biophysical technique such as surface plasmon resonance (SPR), protein−ligand NMR, or even X-ray crystallography is much more achievable compared with assembling and screening a large library in a bioassay. In fact, some of the pioneering work using X-ray crystallography for fragment screening was done at the University of Groningen in the early 1990s.[25] State of the art high field NMR instruments and expertise in other biophysical techniques will often be readily available within world class academic groups, and there have been a number of recent publications from academia in which FBDD methods have been applied to exploratory targets.[26–33]

Since FBDD was last reviewed in this journal in 2004, there has been continued development of fragment-based screening technologies, bringing higher throughput, increased cost effectiveness, and reductions in protein requirements as well as potentially broadening the application to a wider range of therapeutic targets. In this review we will not cover these improved methods in any detail but will briefly outline some of the new technological advances. One important development is that the cryogenic NMR probe has become much more widely available, giving greatly improved sensitivity and therefore improving data quality and throughput of NMR-based screening approaches.[34] Another example is the use of NMR to detect the displacement of "spy" molecules that contain ^{19}F from protein active sites.[35] The introduction of cryogenic probe technology with ^{19}F detection capabilities or the use of capillary NMR probes[36] may in future increase the sensitivity of this method to levels that rival traditional enzymatic assays. We have also seen further refinements in protein−ligand X-ray crystallography,[37] mass spectrometry,[38,39] surface plasmon resonance,[40–42] and isothermal titration calorimetry.[33]

As well as an increase in the number of groups using fragment-based screening methods, the concept of starting from the simplest ligand in a drug discovery campaign has influenced those involved in HTS. There have been a number of reports of the use of high-concentration screening (HCS) or "reduced complexity" screening on compound collections that are a hybrid of a true fragment library and of a typical HTS collection.[12,43–45] In some cases, a somewhat looser set of criteria are applied when constructing fragment libraries than would be used for a fragment set designed for biophysical screening (for example, by allowing the upper molecular weight to approach 350 Da). This has the effect of greatly increasing the available subset of molecules that can be screened within a corporate collection. Screening larger molecules (that are capable of forming more interactions with the target protein and hence delivering higher potency) has the additional benefit that they will, if active, be detectable in a HTS campaign simply by screening at a higher than normal concentration.[45] This strategy is essentially identical to that suggested in 1999 by Teague et al. in which the authors argue that "leadlike" hits (<350 Da) are advantageous as start points for hit-to-lead chemistry compared with the "drug-sized" hits typically delivered from HTS, even if of lower potency.[46,47] Having a looser set of criteria for fragment libraries does, however, raise a number of potential issues, and these are discussed later.

An additional trend, often when there is an absence of structural information or when there is limited chemistry resource to follow up "primary" fragment hits, is to carry out a secondary screen of leadlike analogues in a conventional bioassay. This iteration of SAR generation can lead to identification of analogues with potencies similar to those of typical HTS hits, ensuring that the fragment derived hits are competitive with those emerging from a standard screen and giving confidence in the results from the fragment screening. For example, at Vertex the NMR SHAPES method has been used successfully for 10 years as a complementary approach to HTS and typically 500 follow-up compounds are screened around a promising fragment hit, often leading to the discovery of hits with 5−10 μM potency (70−80% success rate).[48]

2.2. Ligand Efficiency. A valuable concept now widely used for comparing hits across different series and the effectiveness of compound optimization is ligand efficiency (LE). The term LE is defined as the free energy of binding of a ligand for a specific protein averaged for each "heavy atom" (or non-hydrogen atom).[49–51] The number of heavy atoms is termed the heavy atom count (HAC).

$$\mathrm{LE} = -\Delta G/\mathrm{HAC} \approx -RT\ln(\mathrm{IC}_{50})/\mathrm{HAC}$$

Throughout this review the units of LE are (kcal/mol)/heavy atom. If we then consider that an oral drug candidate should have a molecular weight of <500 Da (to fulfill Lipinski's rules) and typically IC_{50} < 10 nM, we can extrapolate that a minimum LE of 0.3 is required in a hit or lead for it to be useful. This value can then be used as a guideline for the LE required in a good fragment, assuming LE cannot be improved during the optimization process.

Kuntz et al. in 1999 were the first to calculate normalized potencies in a similar way and also to suggest that such values would be useful in tracking the potencies of molecules.[52] From the outset, groups carrying out fragment-based discovery were generally monitoring potency vs molecular weight during fragment optimization, but this idea was not more widely adopted until Hopkins et al. introduced the term LE in 2004.[49] Today, not only is it used by practitioners of fragment-based drug discovery,[9,51,53] but it is being adopted more generally by the medicinal chemistry community. The use of LE helps to reduce the seductive influence of increased potency after each iteration of compound design and serves to remind the chemist that consideration of the physicochemical properties of a lead series is equally important. Recently, modified definitions of LE have been proposed,[50,54] and there is also an increased emphasis on tracking increasing lipophilicity with potency.[55] Overall, monitoring of LE provides a conceptual "roadmap" for the fragment optimization process.[51]

2.3. Group Efficiency. A recent evolution of LE is to look at group efficiency (GE). This allows for the estimation of an individual group's contribution toward the overall free energy of binding (ΔG), giving a quick and simple insight into how efficient one modification is over another. This analysis requires the comparison of matched pairs of compounds using a Free−Wilson analysis. The ΔG values can be converted into GE in an analogous manner to LE, GE = $-\Delta G$/HAC, where HAC is the number of non-hydrogen atoms in a particular group. GE ≥ 0.3 indicates that the group is making an acceptable contribution to the compound's potency overall, ensuring maintenance of good druglike properties.[28,56] Figure 1 shows the group efficiencies for various parts of a potent protein kinase B (PKB/Akt) inhibitor and effectively highlights key hot spots for binding with this type of molecule. Matched compounds were used to determine the GE for various parts of the molecule; for example, fragments A and B were used to determine the GE for the methyl group at the 5-position of the pyrazole (Figure 1).

For this approach to be valid, it is important to be sure that the binding modes for the compounds that are being compared

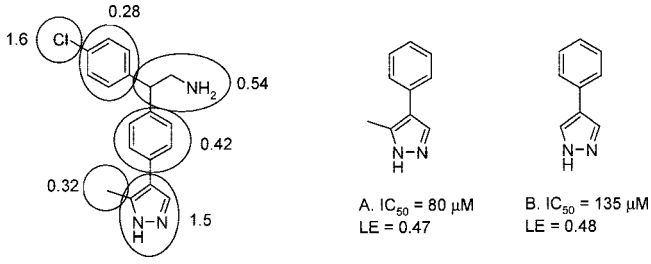


Figure 1. Group efficiencies of a protein kinase B inhibitor and fragment potencies used to derive the GE for the methyl group at the 5-position of the pyrazole ring.

are similar; otherwise, the data could become very misleading. This information can be used to quickly build up which parts of the active site are "hot spots" for gaining affinity and which groups on the inhibitor are the most effective. GE can also be used to elegantly illustrate how the addition of relatively large groups with moderate gains in potency is not necessarily helpful during optimization (for example, the addition of a phenyl group should increase potency by at least 50-fold when GE > 0.3). However, this utility comes with the caveat that the underlying assumption of the group-based additivity of free energies of binding is an approximation and will not always be true.[57]

2.4. Ligand-Lipophilicity Efficiency. Another way of assessing "druggability" is to take into account lipophilicity. This can be done by flagging highly lipophilic compounds that may be deriving a significant proportion of their binding affinity from desolvation. A significant part of protein−ligand binding involves desolvation of the ligand, so the more lipophilic the ligand, the more favorable this process will tend to be. This implies that lipophilic molecules should have an increased chance of binding to any given drug-sized pocket and that lipophilic compounds should be more promiscuous than polar ones as a result. Leeson and Springthorpe have carefully analyzed the effects of lipophilicity on drug failures and concluded that more lipophilic compounds carry significant risks with them into development, especially with respect to increased chances of nonspecific toxicity.[55] It was noted that many pharmaceutical companies are making molecules with relatively high cLogP values (based on the patent literature). They have therefore proposed the use of a new index called "ligand-lipophilicity efficiency" (LLE) as an important guide during lead optimization and as a flag for preclinical candidate selection. LLE is defined as LLE = pIC_{50} (or pK_i) − cLogP (or LogD), and the target LLE for a low nanomolar potency lead was suggested as ∼5−7 or greater. Fragment hits tend to be very polar and water soluble, and the experience at Astex is that the compounds synthesized in order to optimize fragments tend to have lower molecular weight and higher polarity when compared with those outlined in Leeson et al.'s article. For example, in 2006 the median cLogP value for patented molecules from Astex was 2.4, significantly lower than for the equivalent values presented in the paper from some large pharmaceutical companies (AstraZeneca, 3.7; GlaxoSmithKline, 4.2; Merck, 4.0; Pfizer, 3.5). This may then be an additional advantage of using the fragment-based approach.

2.5. Fragment Libraries. There have been a number of discussions in the recent literature of what constitutes a good fragment library. The main considerations are (1) the range of physicochemical properties of fragments to be included, (2) aqueous solubility and quality control, (3) assessment of molecular diversity, (4) chemical tractability of the fragments for follow-up, (5) which chemical functionalities are disallowed, (6) druglikeness of the fragments with respect to precedence of the templates in oral drugs and natural products, and (7) sampling of privileged medicinal chemistry scaffolds.[4,12,19,20,37,45,58–60] Although there are clearly a number of "flavors" of fragment libraries emerging and slightly different definitions of what constitutes a good fragment, these reports are broadly in agreement. In simple terms, a fragment should have a maximum molecular weight of 300 Da (as proposed originally by Astex Therapeutics in its "rule of three"), some complexity filters should be applied (either based on 2D descriptors such as H-bond donors, H-bond acceptors, or rotatable bonds or by using a complexity fingerprint approach), and high aqueous solubility is essential for practical reasons during screening.[61]

Despite this general level of agreement of what a useful fragment library looks like, we saw in the previous section how HCS is being used as one approach to screen fragment libraries using molecules that tend to be at the upper limits of what would constitute a fragment. Indeed, it is our belief that HCS will become very popular in large pharmaceutical organisations because it requires very little change to current best practice and might be expected to deliver high quality hits for more tractable targets. However, the two basic principles of FBDD (the use of very small compounds and very high screening sensitivity) need to be carefully reconsidered when using HCS for fragment libraries. There are two main issues to be addressed. First, as the size of a fragment increases, the number of sensible molecules will grow dramatically, making it much harder to design a diverse library.[1] Additionally, it is now generally accepted that as molecular size and complexity grows, the chances of an H-bonding mismatch or steric clash between the fragment and the target protein rapidly increase, reducing the chance of finding hits.[62] Both of these factors suggest that a much larger library of "leadlike" compounds will be required to achieve a hit rate comparable to that generally observed for small fragments screened using very sensitive biophysical techniques at a higher concentration. Second, at lower concentrations the smallest fragments will only be detectable if they have potency similar to that of the larger, more complex compounds being screened. This means that the individual interactions must be very efficient in the smallest fragments, or the binding information will be lost in the noise of the assay. This last factor is captured by the concept of ligand efficiency, which was discussed earlier.[49]

In considering the first of these two issues (the idea that "chemical space" explodes dramatically as molecular size increases), an assessment of the relative sizes of different libraries that might currently be assembled from commercial sources can be made. Although this analysis does not measure how actual chemical diversity changes with molecular size, it should help assess how thoroughly one can sample chemical space in practice. Figure 2 plots the distribution of four libraries of available molecules that can be purchased from commercial databases. In each case the compounds considered for possible inclusion contained carbon and at least one ring and did not contain inorganic elements or deuterium isotopes. There are many more druglike filters that could additionally be applied, but for the purposes of the comparisons on the chart no further filtering has been used. By use of these simple constraints on a subset of our in-house compound database of commercial samples, 2 255 680 compounds were found to be potentially available. By use of the "rule of three" filters (Ro3 library; MWT ≤ 300, cLogP ≤ 3, number of H-bond donors ≤ 3, number of H-bond acceptors ≤ 3), there are approximately 137 000 possible compounds available in a fairly even distribution across the 7−23 heavy atom range. This distribution is tightly

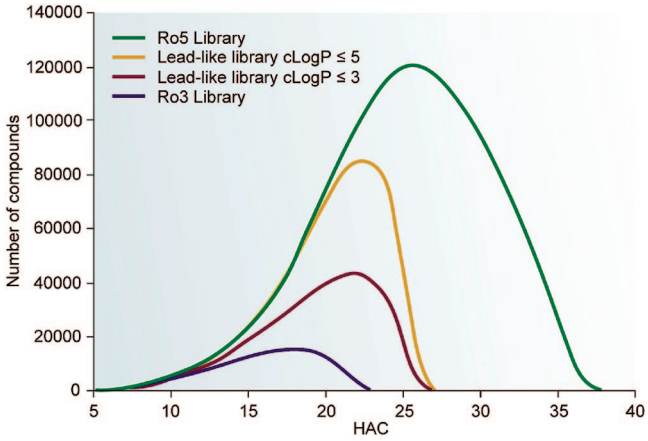


Figure 2. Comparison of the distributions of commercially available compounds that are candidates for different types of screening collection. Twenty-two preferred suppliers were selected. Rule of three (Ro3): MWT ≤ 300, ClogP ≤ 3, Donors ≤ 3, Acceptors ≤ 3. Leadlike: MWT ≤ 350, ClogP ≤ 3 or 5, Donors ≤ 5, Acceptors ≤ 10. Rule of Five (Ro5): MWT ≤ 500, cLogP ≤ 5, Donors ≤ 5, Acceptors ≤ 10. All compounds must be listed as "available" and must contain at least one carbon atom in a ring. The allowed elements are ^{1}H, C, N, O, S, F, Cl, Br, I.

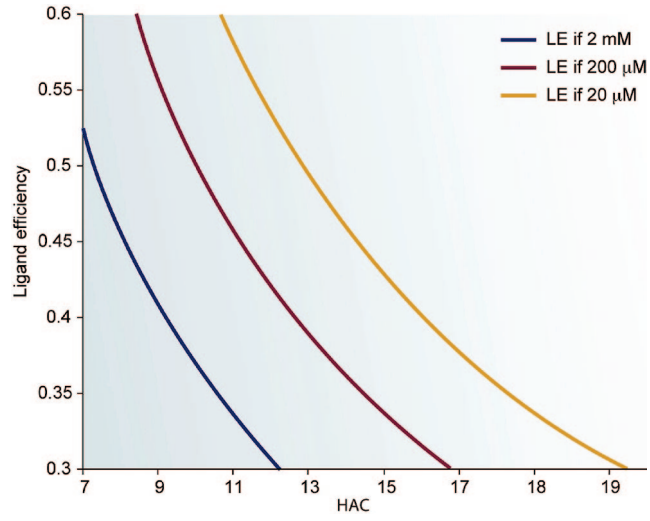


Figure 3. Plots of the number of heavy atoms of a given fragment vs the ligand efficiency that would be required for that compound to be detected as a hit when screened at 2 mM (blue), 200 μM (purple), and 20 μM (orange).

controlled by the restrictions on numbers of donors and acceptors, which serves to limit molecular complexity. Similarly, Siegal et al. have suggested that, after applying more rigorous druglike selection filters than used here, 70 000 "Ro3 compliant" fragments are commercially available from preferred suppliers.[63] By comparision of this with compounds selected using Lipinski's "rule of five" guidelines for oral drugs (Ro5 library; MWT ≤ 500, cLogP ≤ 5, number of H-bond donors ≤ 5, number of H-bond acceptors ≤ 10), there is an even overall distribution either side of 26 heavy atoms and a total of approximately 1 700 000 compounds to choose from. Finally, applying leadlike filters of MWT ≤ 350 and either cLogP ≤ 3 (as originally described by Teague et al.) or cLogP ≤ 5 (according to Lipinski's rules) and also requiring compounds to obey the other rules suggested by Lipinski produce libraries of approximately 400 000 and 700 000 compounds, respectively (lead-like library cLogP ≤ 3 and leadlike library cLogP ≤ 5), with a right-shifted distribution toward members with higher molecular weight.[46]

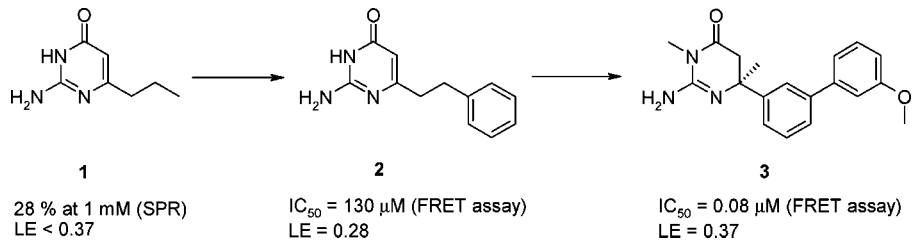
Figure 2 illustrates three main points. First, a molecular weight cutoff alone is a poor start point for defining a library of molecules because without cLogP and complexity filters the distribution of available molecules will be skewed toward the upper mass limit. Second, the available Ro3 library is significantly smaller than the Ro5 and leadlike libraries, suggesting it should be practically more straightforward to create a library of compounds that adequately represents "commercially available diversity" within this area of chemical space than for the other two types of library. Lastly, even a small increase in the upper molecular weight limit (perhaps 50 Da) in a fragment library will dramatically increase the number of available molecules that can be considered. True chemical diversity will actually have massively increased, meaning that the largest fragments within the library will inevitably represent their region of chemical space much less well than the smallest compounds.[12]

To examine the issue of hit detection outlined above, it is useful to again consider LE. Figure 3 plots the HAC of a given fragment vs the LE that would be required for that compound to be detected as a hit when screened at 2 mM (dark blue), 200 μM (purple), and 20 μM (orange). A fragment with a HAC of 12 (MWT typically 160−170 Da) will require a LE of 0.3 or greater to be detected when screened at 2 mM (a concentration that can only be reliably screened using a biophysical method, such as protein−ligand NMR, SPR, or protein−ligand crystallography). As discussed earlier, this is the minimum useful LE required to develop a fragment into a potent molecule that obeys Lipinski's rules. For the same fragment to be detectable at a concentration of 200 μM (for example, in HCS), it would require a LE of 0.42, and any fragments with lower LE would not be detectable. A LE of 0.42 or greater is much less common than a LE of around 0.3 because it generally requires multiple high quality interactions to be present between the ligand and the protein and (depending on the difficulty of the target) may not always be achievable. If the same molecule was screened at 20 μM (in a typical HTS campaign), it would require a LE of 0.53. Such high LE values are rarely observed and may require the protein target to be highly tractable (for example, a kinase enzyme target would be suitable).

The above analysis indicates that fragments with HAC of around 12 or less are unlikely to be detectable in a HTS for all but the most tractable of drug targets, and even in a HCS it is probable that many fragments that are binding to the target will not be doing so efficiently enough to be detected. Furthermore, if we examine the curves, we can see that for very small fragments (HAC = 8 or less) in most cases X-ray crystallography will be required to detect their binding, as only this approach is likely to be reliable in the 2−10 mM binding range. Overall, taking together the LE requirements for fragments to bind at different concentrations with the dramatic increase in availability of compounds as the upper limit of molecular size is increased, we can conclude that in a typical HCS campaign the majority of the binding information will come only from the largest compounds in the screening library. This is because the library is likely to be more highly populated with larger compounds than smaller ones, and in any case only the larger compounds can be readily detected as hits for most targets.

Figure 3 also illustrates that changes in LE are not linear with changes in HAC. This means that as fragments get smaller for any given potency LE increases more quickly, and alternatively as drug sized ligands get larger, LE decreases more slowly. The result of this parabolic mathematical relationship is that LE is very sensitive for small fragments (small changes

Scheme 1. Fragment Evolution of 6-Propyl Isocytosine Leading to a Potent Nonpeptidic BACE-1 Inhibitor



in potency cause quite large changes in LE) but relatively insensitive for larger compounds (LE does not change as noticeably as HAC increases). Overall, therefore, it is important to use LE as a guide for ranking of fragment hits and also to assess the success of optimization of fragments into potent larger ligands but not to overinterpret absolute LE values.

In conclusion, it is our belief that FBDD is most powerful when screening a library of smaller fragments (100−250 Da) using very sensitive biophysical methods at millimolar concentrations. These smaller fragments have the best chance of finding productive interactions with a given target protein, without containing a mismatch or steric clash that would compromise binding,[62] while still delivering sufficiently high LE to be detectable. When larger compounds are used and screened at a lower concentration (such as 200 μM), the results might be disappointing for all but the most tractable targets. This suggests that FBDD may have significant advantages over HTS (and perhaps HCS) when employed against more challenging targets, such as proteases, phosphatases, and protein−protein interactions.

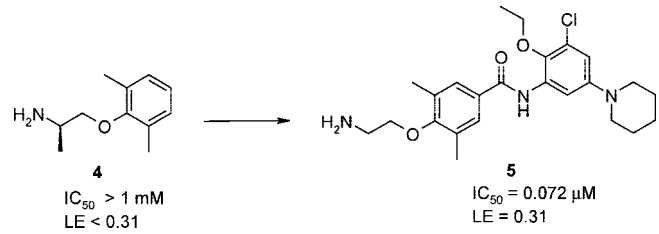
3. Optimization of Fragments

The following section outlines a number of successful strategies for the optimization of fragment hits to leads and in one case to a clinical candidate. Many examples can now be found in the literature, and it is not the intention to list these here but rather to select one or two representative and influential case histories that illustrate the success of each approach.[9,15] Indeed, a very recent review by Alex and Flocco tabulates 62 fragment examples identified for 46 protein targets, illustrating the rapidly gaining popularity of FBDD.[64] However, for 29 of the 62 examples listed the fragments are potent enough to be detected by standard methods; here, we have focused on examples where a FBDD technology is required to identify the fragment hits.

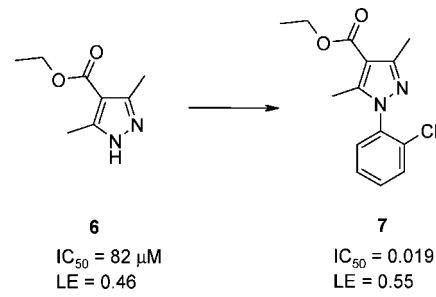
3.1. Evolving Fragments. Fragment binding can generally be improved by substitution at one or more vectors with additional functionality. This "fragment evolution" has proved to be the most popular and effective approach to fragment optimization. Structural information of the fragment hit (X-ray or NMR) is used to design larger molecules that pick up additional protein−ligand interactions resulting in improved affinity for the target. A requirement for success is that the fragment acts as an "anchor" and does not change its binding mode during its evolution to a potent lead.

3.1.1. β-Secretase (BACE-1). The aspartyl protease enzyme β-secretase is considered a very challenging target by the industry, but the biological evidence is compelling that its inhibition will be useful in the treatment of Alzheimer's disease. Mixtures of fragments have been screened using a 1D NMR screening approach, and fragments such as **1** (Scheme 1) were identified with millimolar affinity as determined by surface plasmon resonance (SPR).[65] Protein−ligand X-ray crystallography determined that the fragments were bound through the isocytosine motif to the catalytic aspartic acid residues in the active site. This type of charged bidentate interaction expressed a newly discovered pharmacophore for this class of enzyme.[66] By use of pharmacophore and structure-based drug design approaches, potency was initially improved to the micromolar inhibitor **2**, and then further optimization gave the lead molecule **3** with submicromolar affinity.[67]

Scheme 2. Fragment Evolution of the (*R*)-Enantiomer of the Oral Drug Mexiletine to a Potent uPA Inhibitor



Scheme 3. Fragment Evolution of PDE4 Inhibitors Using Scaffold-Based Drug Design

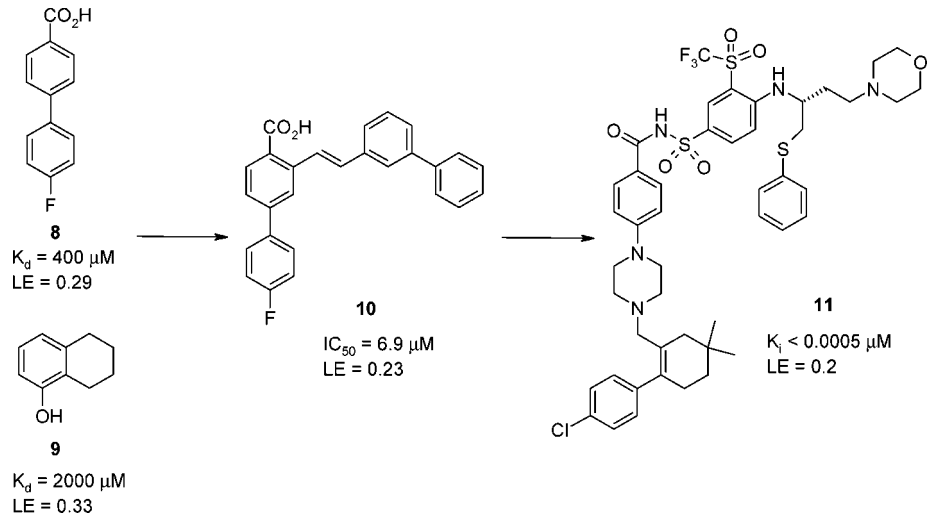


3.1.2. Urokinase (uPA). In a very recent example, the (*R*)-enantiomer of the fragment-sized oral drug mexiletine **4** (Scheme 2) was identified as a hit binding to the active site of the serine protease urokinase (urokinase-type plasminogen activator, uPA) by X-ray crystallographic screening.[68] Despite having potency too weak to be accurately measured, the fragment was optimized to a potent lead molecule **5**, using structure-based design approaches. The lead compound was additionally shown to have promising selectivity vs related proteases and a pharmacokinetic profile similar to mexiletine itself, with good oral bioavailability and long half-life in rat. This example illustrates the value of screening low molecular weight drugs as part of a fragment library.[20]

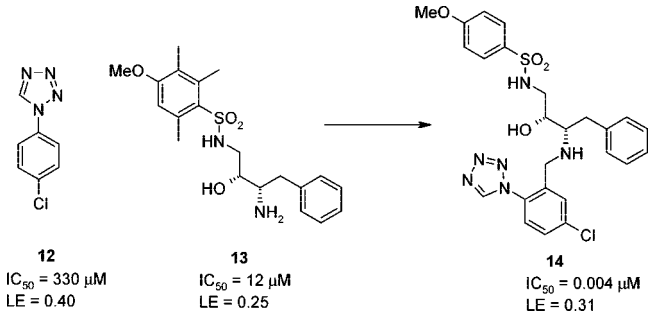
3.1.3. Phosphodiesterase 4 (PDE4). Researchers at Plexxikon have screened a library of around 20 000 scaffold-based compounds (molecular weight range 125−350 Da) against 5 PDE family members using HCS. A follow-up screen using X-ray crystallography identified the pyrazole ester **6** (Scheme 3). After just two rounds of chemical synthesis, a number of low nanomolar potency compounds were discovered, including **7**, equating to a 4000-fold increase in affinity.[69]

3.2. Combining Fragments. In some cases more than one fragment can be detected binding to nonoverlapping regions of

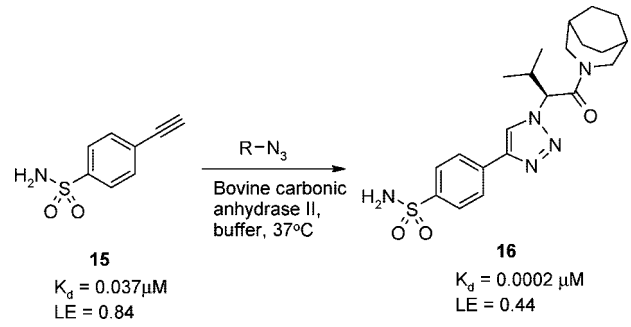
Scheme 4. Fragment Linking for the Bcl-2 Family of Proteins Leading to the Identification of **11**



Scheme 5. Fragment Linking Leading to Potent Non-Amidine Containing Inhibitors of the Blood Coagulation Target Thrombin



Scheme 6. In Situ Click Chemistry Was Used To Identify Subnanomolar Inhibitors of Carbonic Anhydrase, a Zinc Containing Metalloenzyme



a protein. It may then be productive to link such fragments, either directly or through a suitable linker. The challenge has often been identifying a suitable linker strategy that allows both fragments to maintain their original binding modes when combined in the new hybrid ligand and consequently retain acceptable LE.

3.2.1. Bcl-X_L. Lead generation for the protein−protein anticancer target Bcl-X_L was explored using a high-throughput NMR-based method "SAR by NMR".[70] A chemical library of small molecules was screened for their potential to bind to the large highly lipophilic BH-3 binding groove of Bcl-X_L, a Bcl-2 family member. In this way fragments **8** and **9** were found to bind to distinct but proximal subsites within the binding groove of the active site (Scheme 4). By use of NMR derived structural information and knowledge of key binding points for the native substrate (BAK), the two fragments were linked via an alkene functionality and then optimized for potency to give the low micromolar compound **10**. Significant optimization efforts led to the discovery of **11**, an agent suitable for oral dosing, which is currently in phase I/IIa cancer clinical trials (Table 2).

3.2.2. Thrombin. A small targeted library of fragments (selected using virtual screening) was screened by soaking into protein crystals of thrombin, and fragment hits were detected directly by X-ray crystallography.[71] A number of neutral S_1 site binders were identified (Scheme 5), and one of these, fragment **12**, was linked to a larger ligand **13** which spanned the S_2 and S_4 subsites (derived from a virtual screening hit). With this fragment linking strategy, highly potent hybrid inhibitors such as **14** were discovered. The fragment binding mode of **12** is illustrated in Table 1, entry 9 (section 4.2.9).

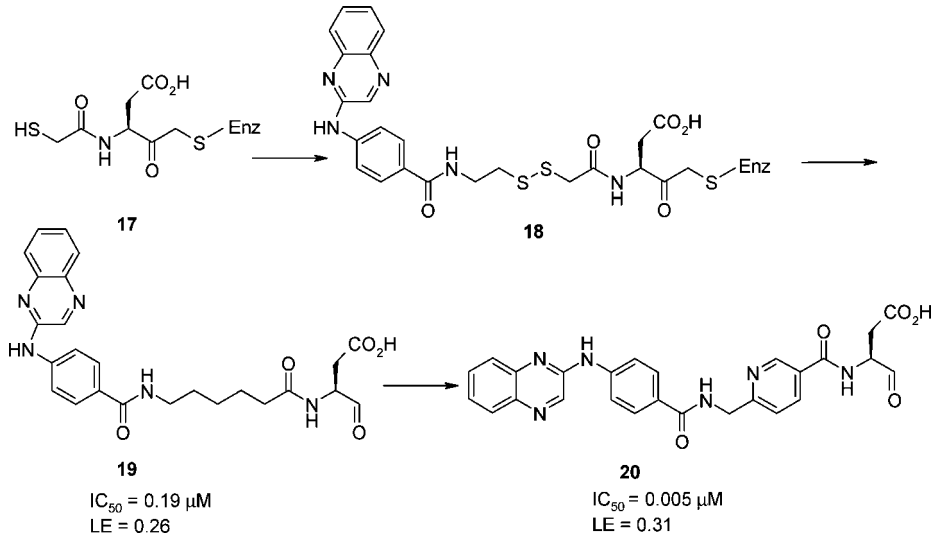
3.3. In Situ Fragment Linking. Fragment linking has been taken a step further by the use of in situ approaches such as dynamic combinatorial chemistry[72] and in particular the use of "click chemistry".[73] Here, there is a requirement for mild chemistry that can take place at room temperature in an aqueous environment in order to generate the inhibitors.

3.3.1. Carbonic Anhydrase. In this approach the protein target of interest is used as a scaffold to bind adjacent fragments containing suitable reactive functional groups. A chemical reaction takes place, linking the two fragments to form an inhibitor in situ in the active site of the protein. The example shown (Scheme 6) used "click chemistry" to identify subnanomolar inhibitors of carbonic anhydrase, a zinc containing metalloenzyme. Aromatic sulfonamides such as **15** were known to strongly coordinate with the Zn^{2+} cation of the enzyme, and the acetylene functional group was then appropriately positioned for combination with complementary azides via a 1,3-dipolar cycloaddition reaction that resulted in the triazole based inhibitors. For example, compound **16** showed a 185-fold improvement in binding affinity over the initial fragment.[74]

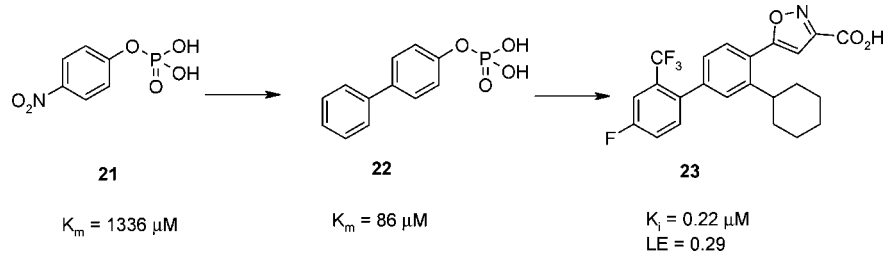
3.4. Fragment Tethering. This strategy relies on the formation of a disulfide bond between a chemically reactive fragment and a cysteine residue in the target protein. Fragments with the greatest affinity for the protein within the vicinity of the cysteine form the most stable disulfide bonds and are readily detected by mass spectrometry.[75]

3.4.1. Caspase-1. A nice example of this tethering approach has been reported for screening and optimization against caspase-1 (Scheme 7). First, a key recognition unit (the aspartyl

Scheme 7. Identification of Caspase-1 Inhibitors Using a Fragment Tethering Approach



Scheme 8. Identification of a Potent Inhibitor of PtpB through Fragment-Based Substrate Activity Screening



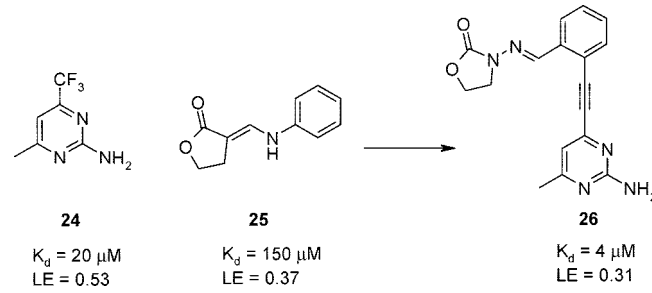
group of the substrate) was covalently linked to the active site cysteine of caspase-1 to give adduct **17**. Next, thiol fragments capable of forming a disulfide bond with this enzyme complex were screened and hits such as **18** were identified by mass spectrometry; these groups were later shown to bind into the P_4 pocket of the enzyme by X-ray crystallography. Replacement of both the disulfide bond with a simple methylene linker and the covalent cysteine binder with a reversibly binding aldehyde resulted in compound **19** with submicromolar affinity.[76] Further optimization by rigidifying the linker unit produced a ligand efficient low nanomolar inhibitor **20**.[77]

3.5. Using Fragments as Substrates. Ellman's group have developed a novel fragment-based screening method called substrate activity screening (SAS) for the efficient development of novel nonpeptidic protease inhibitors.[78,79] Here, the fragments being screened are substrates of the protease and are only later converted into inhibitors. This approach has also been recently modified and employed for the discovery of novel protein tyrosine phosphatase inhibitors.

3.5.1. Protein Tyrosine Phosphatase B (PtpB). A spectrophotometric method was employed to screen a diverse library of *O*-aryl phosphates as potential substrates for the bacterial protein tyrosine phosphatase B.[80] PtpB has potential as a target for the treatment of tuberculosis. Compound **21** was used as a standard and had a K_M of approximately 1 mM. The screen identified the biphenyl analogue **22** as a significantly better substrate (Scheme 8). Systematic modification of the biaryl motif, followed by introduction of a suitable phosphate group isostere, resulted in compound **23**, which is the most potent inhibitor of PtpB reported in the literature to date.

3.6. Using Fragments To Identify New Binding Sites. Protein targets almost always have a "hot spot" within their binding sites, for example, the hinge region within the ATP

Scheme 9. Identification of Second Site Binders in HSP90 by 2D NMR Screening



binding pocket of a kinase enzyme or the metal ion in a matrix metalloproteinase. It is often possible to block this hot spot by binding of one fragment and then probe the resulting protein−ligand complex for a second binding event using a further set of fragments. In this way, fragment-based screening may be used to identify molecules that bind to adjacent binding sites, potentially allowing for a fragment-linking approach to subsequently be employed. In the case of more distant allosteric binding sites being identified, this also gives the option of modulating a protein's action via an alternative mechanism.

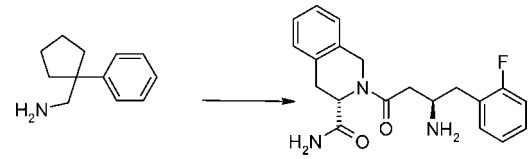
3.6.1. Heat Shock Protein 90 (Hsp90). Huth et al. have identified low micromolar aminopyrimidine based inhibitors of Hsp90 such as compound **24** (Scheme 9) using a fragment-based NMR screening approach.[81] Although this series of compounds could readily be optimized to give nanomolar potency ligands, the lack of novelty of the leads was considered an issue. In an attempt to identify more novel inhibitors, a fragment library was screened against Hsp90 using a 2D NMR method in the presence of compound **24**. In this way, fragment

25 was identified that had $K_d = 150\ \mu M$ in the presence of **24** but $K_d > 5000\ \mu M$ in the absence of **24**, suggesting cooperative binding. With structural data derived from both NMR and X-ray experiments, a fragment-linking strategy was employed to design more potent molecules such as **26** ($K_d = 4\ \mu M$). This elegant example highlights the benefits of using multiple techniques for generating structural data. The X-ray crystal structure suggested a different binding mode for fragment **25** compared with the NMR structure solution, and in this case it was the NMR data that led to the design of the lead compound **26**.

3.7. Computational Approaches. A variety of computational tools have been developed or adapted to support the different phases of fragment-based drug discovery programs, and this is a rapidly developing area. The first step in FBDD is to develop libraries of fragments that will be subsequently screened against the target. The principle considerations were touched on in section 2.5, and most approaches have used chemoinformatics and modeling tools as the starting point to generate fragment libraries. The computational deconstruction of drugs into their constituent fragments is one of the most commonly used methods to build fragment libraries.[82] Lepre and colleagues from Vertex Pharmaceuticals were one of the first groups to use this approach and generated a library containing less than 200 fragments, which they called a SHAPES library, for NMR screening.[83] Similarly, a retrosynthetic combinatorial analysis procedure (RECAP) has been used by Lewell et al. to identify recurring fragments from known drugs.[84] Recently, Kolb and Caflisch have developed a computer program called DAIM (decomposition and identification of molecules), which is able to disassemble molecules into mainly rigid fragments. DAIM, in conjunction with docking protocols, has been successfully applied to identify inhibitors of β-secretase (BACE-1).[85]

Computational methods are also being widely used to try to identify potential hits from virtual fragment libraries. To date, structure-based computational methods have been the most successful in this field. It can be difficult to generate good SAR for early fragment hits because they exhibit such low binding affinity that is then difficult to detect and compare reliably in a biological assay. As a result of this, successfully predicting the binding mode of a fragment bound to its target, when this information is not available, is crucial in order to develop strategies to evolve a hit into a more potent lead molecule. Molecular docking has been successfully used for a number of years to predict the binding modes of druglike compounds, and more recently, this methodology has been applied to dock fragment-like molecules.[86] A typical work flow in FBDD consists of docking a fragment into the binding site of interest, choosing the best orientation and then using this as a starting point for the attachment of substituents with the aim of targeting a new area within the binding site where supplementary interactions might be made. A number of SBDD tools have been developed with this work flow in mind, and these methods have been recently reviewed.[59,87] There are some excellent examples in the literature in which docking approaches have identified virtual fragment hits that have helped to develop inhibitors for a range of different targets that include thrombin,[88] factor Xa,[89] cathepsin D,[90] TGT,[91] and CDK4.[92] However, the low molecular weight and low complexity of fragments have highlighted some limitations in docking methodology. Often, scoring functions are not able to energetically distinguish native from irrelevant poses,[93] and pharmacophoric constraints need to be applied to improve results.[94] Moreover, most of the scoring functions used in molecular docking contain a number of crude approximations of the factors involved in binding and have been optimized for druglike ligands. Not surprisingly then, they do not perform well with fragment-like molecules. Finally, scoring functions are often dependent on ligand size; therefore, virtual fragment libraries should preferably contain molecules of similar size and numbers of functional groups in order to demonstrate good enrichment in a virtual screening experiment.[94] Despite these issues, the success of biophysical screening in FBDD has encouraged the modeling community to try to develop new and more reliable tools with the aim of improving the modeling of fragment binding for virtual screening. Better treatment of solvation and electrostatics[85] and the use of grand canonical Monte Carlo methods to calculate binding free energies[95] have all shown promise and offer new directions in fragment modeling. Two recent examples that illustrate how computational methods have been used for FBDD and in which new potent inhibitors have been discovered are given below.

Scheme 10. Identification of Potent DPP-IV Inhibitors Using in Silico Fragment-Based Screening



27 $IC_{50} = 40\ \mu M$, LE = 0.46

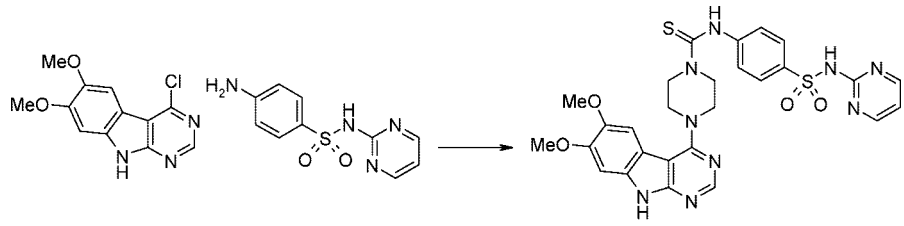
28 $IC_{50} = 0.023\ \mu M$, LE = 0.40

3.7.1. Dipeptidyl Peptidase IV (DPP-IV). Rummey et al. used an in silico docking approach to identify fragments that bound within the S_1 pocket of DPP-IV.[96] A fragment database of approximately 10 000 small primary aliphatic amines was assembled and docked using FlexX. A range of β-phenethylamine based compounds were identified, and fragment **27** (Scheme 10) was chosen for optimization. X-ray crystallography confirmed that the phenyl ring of the fragment was located in the hydrophobic S_1 pocket that accommodates a proline in the substrate. The amino group was shown to form multiple hydrogen bonds to three acidic residues. When this was used as a start point, structure-based optimization resulted in the discovery of a series of potent DPP-IV inhibitors, such as **28**.[97] Another example of the fragment-based discovery of DPP-IV inhibitors is given in Table 1, entry 4, which shows the crystal structure of a substrate-based fragment bound to DPP-IV. Its subsequent optimization is illustrated in part ii of Figure 4.

3.7.2. Aurora A Kinase. Warner et al. screened a virtual fragment library of ~70 000 compounds (including a limited number of known kinase inhibitor fragments) against a 3D homology model of aurora A kinase.[98] Subsequent structure-guided optimization was then carried out using a published crystal structure (1MQ4) from the PDB. The known kinase scaffold **29** (Scheme 11) was identified in silico using the scoring function LUDI and was then confirmed to have inhibitory activity against aurora A ($IC_{50} = 3\ \mu M$). Similarly, compound **30** was proposed to bind in the phosphate binding pocket ($IC_{50} = 21\ \mu M$). A fragment linking strategy was used to give compound **31**, a nanomolar potency inhibitor and a useful starting point for further medicinal chemistry optimization. However, this example highlights the difficulty of linking two relatively ligand efficient fragments together, as the resulting larger molecule has a significantly lower LE and high MWT.

3.8. Deconstructing Leads into Fragments. Taking a lead molecule, breaking it into key fragments, and identifying where they bind can provide a valuable source of structural informa-

Scheme 11. Virtual Fragment Library Screening against Aurora A Kinase



tion. At Astex, we have routinely applied this approach to derive focused fragments for targets using pre-existing lead series from the medicinal chemistry literature. A number of other groups have also explored the idea of fragmenting known drug-sized ligands.[28,53,99–101] In particular, Hajduk has reported an analysis of the deconstruction of 18 highly optimized inhibitors into successively smaller component compounds and fragments.[53] Perhaps unexpectedly, the fragments and final compounds were found to have similar LEs, suggesting that it is possible to maintain the LE of the starting ligand during the optimization of fragments. In fact, at Astex our experience is consistent with these observations, and any fragment considered for optimization will have a minimum LE of 0.3 in order that an optimized high potency lead will have a molecular weight of <500 Da. This criterion would only be relaxed for very challenging targets with borderline druggability such as protein−protein interactions, targets that are now increasingly being considered for FBDD approaches.[102] In our experience it is not reasonable to expect LE to increase significantly during the fragment optimization process unless the LE of the starting fragment can be immediately improved by optimization of its binding mode without increasing (or even by reducing) its size.

Babaoglu et al. have deconstructed a 1 μM potency β-lactamase inhibitor into four small, low affinity fragments.[99] They obtained experimental binding modes for all the fragments (X-ray crystal structures), but only one bound in the manner predicted from the original inhibitor. One fragment bound in a new pocket, two via a novel binding mode within the existing pocket, and only the molecule with slightly higher complexity was found to bind in the same manner as the original compound. This report has been much debated by those engaged in FBDD and has cast some doubts on how reliable the binding mode of a fragment will be during its subsequent optimization. One possible explanation is that only one of the fragments contained the key recognition features required for optimization to this larger inhibitor,[103] another that the starting inhibitor was not optimal.[14] A third possibility is that in some cases it may not be possible to break a potent inhibitor down into very small fragments because synergy between two nonadjacent binding interactions is required for efficient binding. If this is the case, it would follow that one might not expect deconstruction of a potent lead to always derive high quality fragments. In contrast, it has been our observation at Astex that, when starting from efficient fragment hits, a fragment both maintains its interactions and can be used to readily derive a potent lead during its optimization. In the next section we look in more detail at how representative fragments bind to their protein targets and we consider how a number of these fragments compare with larger, more optimized molecules derived from them. In doing so, this question of how reliably fragments can be optimized with maintenance of their original binding mode will be further examined.

4. Fragment−Protein Complexes

In this section we review 12 representative fragment−protein complexes available in the PDB. Here, we have attempted to illustrate the types of interactions typically observed between fragments and their target proteins and have selected examples that highlight some of the issues and opportunities with FBDD.

4.1. Selection of Representative Fragment−Protein Complexes. The Protein Data Bank is an excellent source of information about how ligands interact with proteins (www.pdb.org). Various groups have used this data to implement, test, and improve computational tools for structure-based drug design.[14,104–108] Recently, Hartshorn et al. have published a procedure to extract, analyze, and classify protein−ligand X-ray crystal structures from the PDB.[109] This process delivered a set of clusters of protein−ligand complexes, where each cluster included only high-quality PDB structures (high resolution and optimal electron density around the ligand). In addition, most of the selected complexes were associated with targets of relevance to drug discovery or to agrochemicals and contained only ligands that obeyed Lipinski's rules. This high-quality subset of the PDB (HQPDB) offers an ideal starting point to review public domain structures that contain fragment-like ligands. To the best of our knowledge, this type of analysis has not been done before.

A simple way to extract relevant fragment-containing structures from this HQPDB subset was to apply a ligand MWT cutoff of 300 Da and to exclude ligands that did not contain at least one aliphatic or aromatic ring. After these filters were applied to the 85 clusters in the HQPDB, only 45 of them remained populated. All populated clusters were next inspected using a graphical window containing AstexViewer in order to remove solvent-like molecules, fragments not bound within the active site of the protein in question, and other "false-positive" fragments originated by connectivity errors in the PDB file.[110] Finally, 12 diverse representative protein−ligand structures were selected for the scope of this review. In particular, an effort has been made to represent a broad range of proteins and also to select examples that illustrate the range of interactions seen in the broader context of the public domain fragment−ligand complexes that were inspected.

4.2. Description of 12 Representative Fragment−Protein Complexes. Summarized in Table 1 is an illustration and the associated data for each fragment−protein complex being considered. Each entry contains the 2D structure of the ligand with associated MWT, binding affinity, and LE, a 3D representation of the ligand in the binding site, and a detailed description of the binding mode. The entries are ordered by submission date of the X-ray crystal structure in the PDB database (only structures deposited after August 11, 2000, have been included). Additionally, Figure 4 contains schemes for examples where potent inhibitors in the same chemical series

Table 1. Representative Fragment−Protein Complexes Selected from the PDB

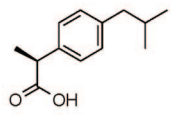
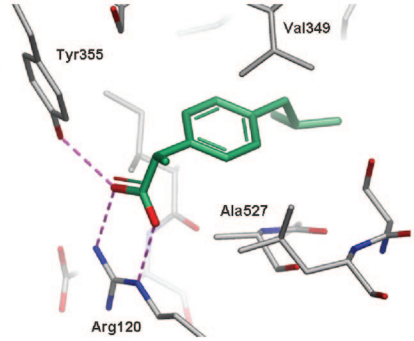
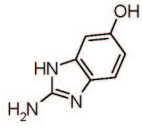
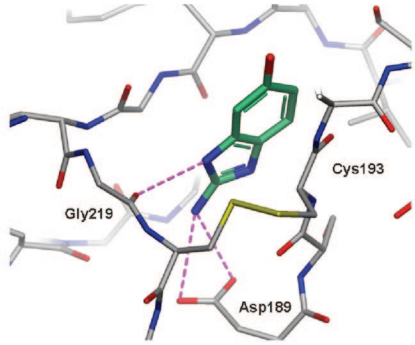
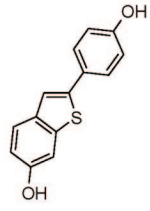
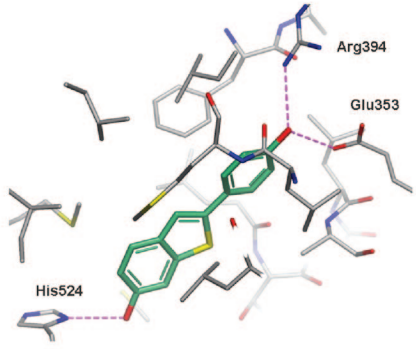
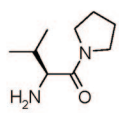
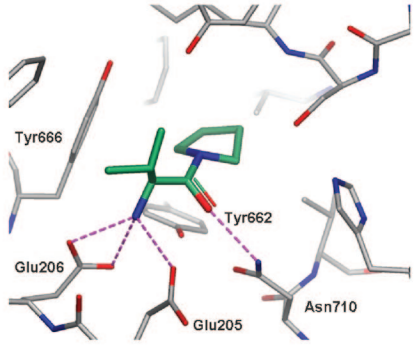
Entry	Ligand	Complex (PDB code)	Description of the binding mode
		COX-1 (1EQG)	
1	 MW = 206 IC_{50} = 2.6 μM LE = 0.51		The carboxylate forms a network of H-bonds to Arg120 and Tyr-355. The phenyl ring is in van der Waals contact with Val349 and Ala527. The distal end of ibuprofen lies in a large hydrophobic cavity. As a consequence, none of the atoms in the isobutyl group are in van der Waals contact with the enzyme.
		uPA (1FV9)	
2	 MW = 149 IC_{50} = 200 μM LE = 0.46		The 2-aminobenzimidazole binds deep into the S_1 pocket. The 2-amino group forms hydrogen bonds to the side-chain carboxylate of Asp189 and the N1 imidazole nitrogen forms a hydrogen bond to the backbone carbonyl of Gly219. The inhibitor is also stacking against the backbone of Cys193.
		ERα (1GWQ)	
3	 MW = 242 EC_{50} = 1.4 μM LE = 0.47		The ligand binding site of estrogen receptor alpha is characterised by a lipophilic cavity with hydrogen bond donors and acceptors at the far ends of it. The arylbenzothiophene scaffold forms lipophilic contacts with the hydrophobic part of the binding site and the phenolic groups form hydrogen bonds to Glu353, to Arg394 and to His524.
		DPPIV (1N1M)	
4	 MW = 170 K_i = 2.0 μM LE = 0.65		The charged amino group of the fragment forms hydrogen bonds to Glu205 and Glu206. The pyrrolidine moiety is buried in a hydrophobic pocket formed by Tyr662 and Tyr666. The carbonyl oxygen forms a hydrogen bond to Asn710.

Table 1 (Continued)

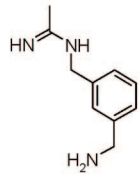
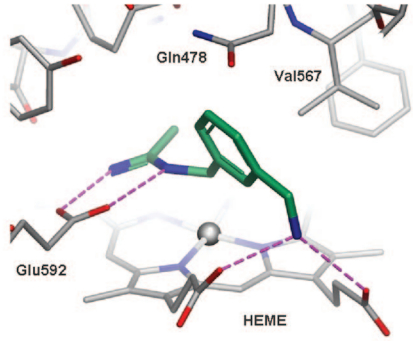
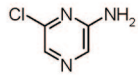
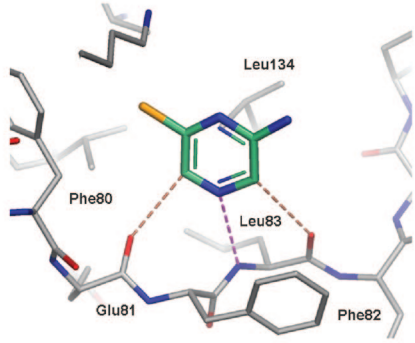
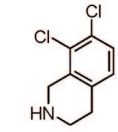
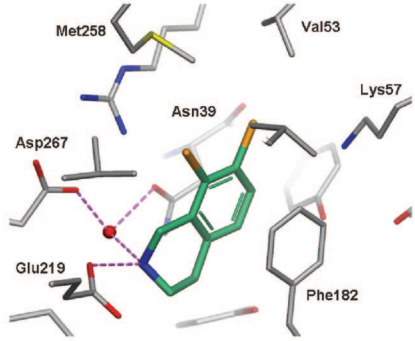
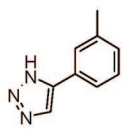
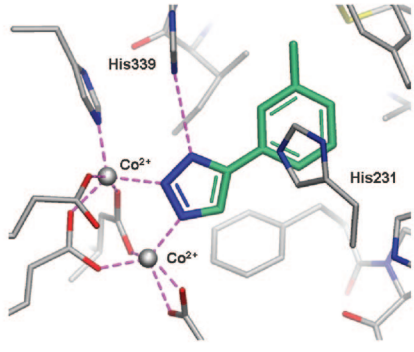
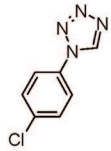
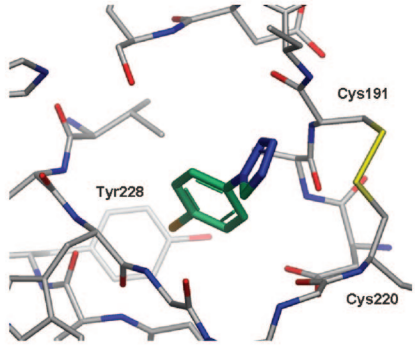
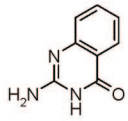
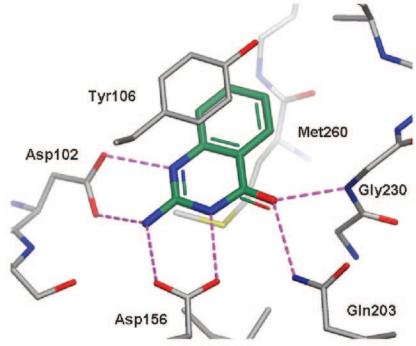
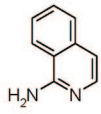
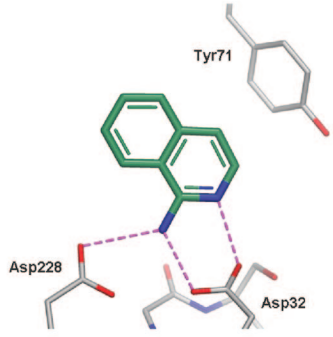
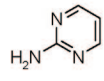
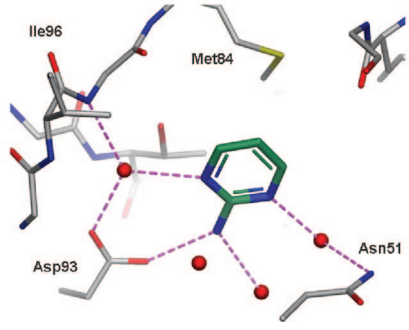
Entry	Ligand	Complex (PDB code)	Description of the binding mode
5	 MW = 177 K_i = 7.3 μM LE = 0.54	**n-NOS (1QWC)** 	The amidine group stacks on the top of the heme and also forms hydrogen bonds with the carboxylate oxygen atoms of Glu592. The benzyl ring is positioned above the heme and forms van der Waals contacts with Val567 and Gln478. The 3-aminomethyl side chain forms salt bridges with both propionic acid groups of the heme.
6	 MW = 130 IC_{50} = 350 μM LE = 0.59	**CDK2 (1WCC)** 	The fragment forms only one hydrogen bond with the protein, between the pyrazine nitrogen and the amide NH of Leu83. The adjacent aromatic C-H's on the pyrazine also form favorable electrostatic interactions (orange dotted lines) with the backbone carbonyl oxygens of Leu83 and Glu81. The fragment forms additional hydrophobic interactions with the side chains of Ala31, Phe80, Phe82 and Leu134.
7	 MW = 202 IC_{50} = 0.003 μM LE = 0.97	**PNMT (1YZ3)** 	The aromatic ring of the tetrahydro-isoquinoline scaffold binds into a narrow hydrophobic cleft between the side chains of Asn39 and Phe182. The ring nitrogen interacts directly with Glu219 and indirectly *via* water mediation with Asp267 and Asn-39. The chlorines make van der Waals contacts with Val53, Lys57 and Met258.
8	 MW = 159 IC_{50} = 0.018 μM LE = 0.88	**MetAp 2 (2ADU)** 	The active site cobalt ions forms key binding interactions with two triazole nitrogens. This tight coordination (interatomic distance 2.0 Å) probably accounts for much of the affinity of this fragment. The third triazole nitrogen engages in a hydrogen bond to His339, while the phenyl ring forms an edge-to-face contact with His231.

Table 1 (Continued)

Entry	Ligand	Complex (PDB code)	Description of the binding mode
		Thrombin (2C90)	
9	 MW = 181 IC_{50} = 330 μM LE = 0.40		The chlorophenyl group binds deeply in the S_1 pocket with the chlorine atom located above the aromatic ring of the Tyr228 side chain. The tetrazole ring makes close contact (3.6 Å) with the sulphur atom of Cys220 in the $S_{1\beta}$ pocket.
		TGT (1S39)	
10	 MW = 161 IC_{50} = 2.1 μM LE = 0.65		The charged aminoquinazolinone forms an extensive network of polar interactions with the protein. All donor and acceptor functionalities in the fragment are involved in hydrogen bonds with different residues (Asp102, Asp156, Asn203 and Gly230). Moreover, the heterocycle is intercalated between the phenolic side chain of Tyr106 and the side chain of Met260.
		BACE–1 (2OHK)	
11	 MW = 144 % inhib = 30-40% at 1mM LE ~ 0.33		The amino group binds between the side chains of Asp32 and Asp228 forming hydrogen bonds. The protonated isoquinoline nitrogen forms an additional hydrogen bond with the side chain of Asp32. The isoquinoline also forms an edge-to-face aromatic interaction with the side chain of Tyr71.
		HSP90 (2JJC)	
12	 MW = 95 Activity not detectable		The aminopyrimidine binds to HSP90 through the formation of four hydrogen bonds. Only one of these is directly to the protein, from the 2-amino group to the carboxyl side chain of Asp93. The other three hydrogen bonds are through a network of conserved water molecules present in the binding site. The aminopyridine ring is in van der Waals contact with the side chain of Met84.

have been reported; in each case the advanced lead is colored according to the moiety that originated from the starting fragment. In some cases the leads were developed from the inhibitors using FBDD, but in others the fragment was identified

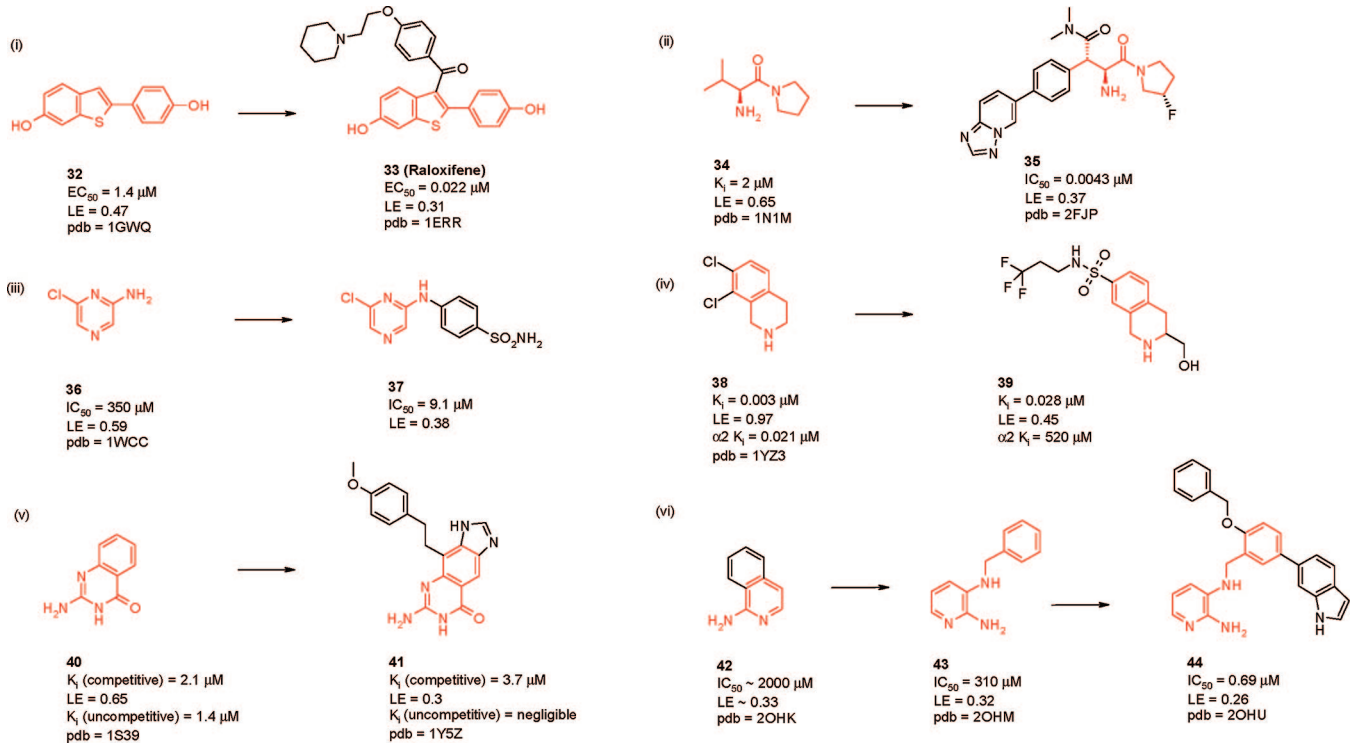


Figure 4. Examples of fragment-sized molecules available in the PDB and their associated drug-sized leads. In some cases the fragment information was used directly to develop the lead, but in other cases the fragment information was only available retrospectively and the lead was developed by standard approaches (see text): (i) ER, (ii) DPP-IV, (iii) CDK2, (iv) PNMT, (v) TGT, (vi) BACE-1.

later by lead deconstruction. Here, we also describe some of our observations for each of the examples, and a number of more general conclusions are discussed in section 5 of this review.

4.2.1. Cyclooxygenase-1 (COX-1). Entry 1 contains the structure of the active enantiomer of ibuprofen complexed with COX-1. Ibuprofen is an example of a marketed oral drug having fragment-like properties. The carboxylate group plays a key role in the recognition of the molecule within the COX-1 binding site, while the shape and the size of the lipophilic group are thought to be important in allowing the molecule to pass through the cyclooxygenase channel itself rather than being critical for affinity.[111] This example illustrates the value of using historical drug molecules and also simplified scaffolds derived from known drugs for fragment screening.

4.2.2. Urokinase (uPA). Entry 2 shows an example of an aminobenzimidazole fragment bound to urokinase. Hajduk et al. used NMR-based screening to identify this novel class of urokinase inhibitors from a library of more than 3000 compounds.[112,113] X-ray crystallography was subsequently used to confirm the binding mode of the 2-aminobenzimidazole hit. This is one of the earliest reported examples of FBDD where a crystal structure of the fragment has been deposited in the PDB. The fragment forms a number of H-bond interactions deep within the S_1 pocket of the protease. The fragments in this entry and in entry 1 are both charged and likely to have high aqueous solubility that would facilitate fragment screening.

4.2.3. Estrogen Receptor α (ER). The ligand in entry 3 of the table is a fragment **32** of the ER antagonist raloxifene, **33** (Figure 4i).[114] The benzothiophene is a rigid scaffold of a suitable length to target two distal polar regions of the binding site with hydroxyl groups. It also offers good growing points to other parts of the active site. The raloxifene structure (not shown; PDB code 1ERR) indicates that the introduction of the large substituent at the 3-position of the benzothiophene altered the binding mode compared with the fragment **32** but that the phenol-binding "hot spot" within the active site is engaged in the same manner.[115] These data suggest that smaller, low potency, phenol based fragments would be expected to bind to the receptor if raloxifene were to be deconstructed, as outlined is section 3.8.

4.2.4. Dipeptidyl Peptidase IV (DPP-IV). Entry 4 describes a very efficient substrate based fragment bound to DPP-IV.[116] This fragment **34** has been evolved into larger and more potent inhibitors such as **35** (Figure 4ii) (a related example was also given in section 3.7.1).[117] X-ray crystal structures show that advanced compounds such as compound **35** (PDB code 2FJP) conserve the original binding mode of the fragment.

4.2.5. Neuronal Nitric Oxide Synthase (n-NOS). Entry 5 shows a fragment bound to the catalytic site in the oxygenase domain of n-NOS.[118,119] This relatively small pocket is an example of a good binding site for fragment-like molecules because it has a volume similar to that of the fragment. To date, the PDB contains 17 NOS structures with nonpeptidic fragment-like molecules bound to the catalytic site in the oxygenase domain. All of these fragments interact with the heme group through the formation of stacking interactions and/or by chelation with the iron atom within the heme itself. The fragment in entry 5 is interesting not only because it binds to this small pocket but also because it offers good vectors to target the area outside the catalytic site region, where it is known it is possible to modulate the selectivity profile of the molecule for the various NOS isoforms. When there are a number of fragments available that bind in a similar manner, choosing examples that allow ready access synthetically to other regions of the active site of interest is a useful strategy for fragment evolution.

4.2.6. Cyclin Dependent Kinase 2 (CDK2). Entry 6 shows 2-amino-6-chloropyrazine **36** bound to the hinge region of CDK2.[37,120] The fragment picks up interactions similar to those of the native ligand ATP. The compound has low affinity (IC_{50}

= 350 μM), but because of its low MWT, it has good ligand efficiency (LE = 0.59). Substitution of the aminopyrazine **36** with an arylsulfonamide gave only a modest jump in activity to compound **37** (IC_{50} = 9.1 μM) and a reduction in LE (0.38), even if X-ray crystallography confirmed (unpublished data) that compound **37** retained the binding mode observed in the original fragment **36** (Figure 4iii). This is hard to rationalize, as good hydrophobic contacts are made between the aryl group and hydrophobic residues at the protein surface and the binding of the pyrazine portion of the molecule is identical to that of the starting fragment. An additional H-bonding interaction between the NH_2 of the sulfonamide and Asp86 is observed, but this is at the expense of breaking an intramolecular salt bridge between Asp86 and Lys89. This latter point may explain the modest jump in activity. Optimization of this series at Astex was subsequently halted in favor of other fragments in which LE was more easily maintained during optimization and which eventually led to two clinical candidates (Table 2), illustrating that it is important not to focus merely on increasing potency but instead on maintaining LE for successful fragment optimization.

4.2.7. Phenylethanolamine *N*-Methyl Transferase (PNMT). Entry 7 shows the X-ray crystal structure of a very active and efficient tetrahydroisoquinoline (THIQ) based inhibitor **38** bound to the active site of PNMT, a key enzyme in the biosynthesis of epinephrine. Grunewald et al. used this fragment as a lead compound to design in selectivity over the α2-adrenoceptor and modulate the physiochemical properties of the series to improve CNS penetration (Figure 4iv).[121] Compound **39** had good affinity for the target enzyme and was found to be 19000-fold selective over the α2-adrenoceptor. The X-ray crystal structure for **39** is not available in the PDB; however, the structures of closely related analogues (e.g., PDB codes 2G8N and 2G71) retained the binding mode of the original THIQ core. This example illustrates how having high LE leaves scope to affect the druglike properties of a molecule, giving "molecular weight head room" to facilitate the lead optimization process for a given series of inhibitors.

4.2.8. Methionine Aminopeptidase 2 (MetAp2). Entry 8 is an example of a fragment acting as a metal chelator.[122] The strong interaction between the 1,2,3-triazole and the two cobalt cations confers a high degree of LE to the fragment. Considering its small size and high LE, aryltriazole based inhibitors of this type may provide a promising template for the design of new inhibitors for methionine aminopeptidase, a target relevant in oncology. Indeed, related triazoles, which show similar interactions with the active site cobalt ions, have recently been disclosed.[123] Metalloproteinases are another class of enzymes for which FBDD is ideally suited because of the high LE available from interacting with metal ions important in enzyme catalysis.

4.2.9. Thrombin. Entry 9 shows a fragment bound to the S_1 pocket of thrombin. It is interesting to note that the ligand has good LE, even if binding is driven predominantly by hydrophobic interactions.[71] The optimization of this fragment via a linking approach has been described in section 3.2.2. This example indicates that fragments need not have multiple polar interactions to have good LE, especially if the target's active site has deep and well defined pockets on its surface.

4.2.10. tRNA-Guanine Transglycosylase (TGT). Entry 10 shows a 2-aminoquinazolinone fragment bound to the catalytic site of TGT. In contrast to the previous example, the fragment forms six hydrogen bonds in addition to good lipophilic interactions with the enzyme, resulting in a highly ligand efficient compound.[124] The aminoquinazoline has been developed into larger leadlike molecules using SBDD.[125] Figure 4v shows an aminoquinazoline derivative **41** that retains the binding mode of the original fragment **40** (compare PDB entries 1S39 and 1Y57). However, the side chains of **41** are not used to increase the binding affinity but instead to modulate the enzyme kinetic profile of the series; the original fragment **40** is a noncompetitive TGT inhibitor, whereas the evolved molecule **41** is a competitive TGT inhibitor. This is another example of how good LE can leave room for optimization of other desirable properties, as well as high potency.

4.2.11. β-Secretase (BACE-1). Entry 11 shows an aminoisoquinoline fragment bound to the two catalytic aspartic acid residues of β-secretase (BACE-1). This type of charged bidentate interaction represents a new pharmacophore for this class of enzymes.[66] With this information, **42** was developed into alternative fragments such as compound **43** using virtual screening by docking using an aminopyridine/amidine pharmacophore derived from the structure 2OHK. SBDD was then applied to design inhibitor **44** that accessed both the S_1 and S_3 pockets of the protease (Figure 4vi).[126] Compound **44** conserved the binding mode observed in fragment **43** (see PDB entry 2OHU). It is interesting to note that the relative position of Asp32 and Asp228 in β-secretase resemble the positions of Asp156 and Asp102 in TGT (entry 10). The binding mode of isocytosine fragments discovered for β-secretase are described in section 3.1.1 and are quite reminiscent of the interactions made by the aminoquinazolinone fragment in TGT. This is a good example of how fragments have the potential to form similar interactions in different and unrelated targets.

4.2.12. Heat Shock Protein 90 (Hsp90). Entry 12 shows 2-aminopyrimidine bound in the ATPase domain of HSP90. This structure is particularly noteworthy because the ligand makes only one hydrogen bond directly with the protein and three additional hydrogen bonds through conserved water molecules. The fragment has undetectable binding affinity because it is so small. Despite this, however, this fragment can still be optimized and larger analogues with submicromolar potency and good LE have been reported in which the aminopyrimidine fragments form identical interactions with the protein (see section 3.6.1).[81] This illustrates how even very small fragments serve as useful hits and can provide valuable binding information.

4.3. Summary. In this section we have used HQPDB to obtain a selection of high quality protein structures that contain fragment-like ligands. Twelve representative examples were selected in order to highlight some general aspects and properties of fragments. Below we report a short summary of our findings:

(1) The binding mode of fragments is often driven by good quality polar interactions and also by good shape matching with the binding site. However, fragments can sometimes also bind purely through hydrophobic contacts and still have good LE.

(2) Fragments can generally be optimized into potent inhibitors while maintaining the original binding mode of the starting hit.

(3) Fragments have the potential to be more promiscuous binders than larger molecules.

(4) Ligand efficient fragments can be evolved into relatively small lead molecules (with high LE), leaving a "molecular weight window" to allow for optimization of other properties such us PK or selectivity (with some consequent reduction in LE).

5. Discussion and Conclusions

In this review we have attempted to examine the trends and developments in FBDD and fragment screening over the past 4

years. To date, fragment screening has been applied in three main ways. First, its use as a primary screening tool has been well documented with many examples being reported, some being outlined earlier. This approach is popular within academia and biotechnology companies. Second, FBDD is being used successfully to identify hits when a HTS campaign has failed to yield useful results against difficult targets.[67] Third, it is starting to become more common within large pharmaceutical companies to run FBDD in parallel with HTS campaigns.[12] This last trend is due to the undoubted success of the methodology for many targets. However, there is still skepticism within the pharmaceutical industry that the optimization process to give nanomolar potency leads from very low affinity hits can be more broadly and routinely achieved. The examples discussed herein and in other reviews indicate that fragment-based approaches have been successful for a wide range of targets, and our experience here at Astex is that if FBDD identifies a number of hits with good LE (>0.3), then optimization of at least one of these series to potent and efficient low nanomolar potency leads will generally follow, provided that there is reliable access to protein−ligand structural information.

The selected examples in section 3 illustrate both the broad applicability of FBDD and the rate of new developments in the underlying fragment binding detection technology. Fragment evolution has been by far the most successful method of fragment optimization. It is conceptually the most straightforward and particularly when allied with a high degree of structural information (e.g., from NMR or X-ray crystallography) gives the medicinal chemist a valuable advantage in the validation and subsequent optimization of a hit. The fragment itself binds to the "hot spot" in the active site, with structural information being used to guide iterative cycles of design to identify further positive interactions between the ligand and protein. Inhibitors of many tractable targets, such as kinases with their well defined ATP binding sites, have been discovered by this approach. However, far more challenging targets, for example, the aspartyl protease BACE-1 for the potential treatment of Alzheimer's disease, have also been successfully targeted (section 3.1.1). In this case many groups had failed to find tractable nonpeptidic lead series using traditional HTS, whereas FBDD proved to be a very effective alternative. Where more than one fragment has been observed to bind to different regions of a target protein, a fragment-linking strategy can be applied. This methodology has been used to identify very high affinity lead molecules, for example, by Abbott to design a Bcl-X_L inhibitor now in clinical trials. However, very precise linking of the two fragments must be achieved in each case to give the expected benefits of superadditivity, and so far, the LEs of inhibitors have generally been lower than those of the starting fragments.[54] We have seen many other innovative developments over the past 4 years that have helped this area progress rapidly, including novel approaches to HCS such as substrate activity screening, further applications of fragment tethering, improvements in virtual screening, and the harnessing of the chemospecific reactivity of fragments using in situ click chemistry.

Section 4 illustrates, for those remaining doubters of the value of FBDD, that in fact fragments have been with us for many years (but have just not been recognized as such) and also that they do not differ in the types of interactions they form with their targets compared to larger, more potent ligands (except that they generally form fewer of them). Indeed, we can see from the binding of ibuprofen (entry 1 in Table 1) that the compound looks grossly similar in the number of interactions and van der Waals contacts as many of the other examples given, and this compound is a licensed drug. An analysis of our in-house oral drug database containing 1195 licensed oral drugs (an updated version of the database described by Vieth et al.) indicates that 211 drugs (17.7%) obey the "rule of 3" discussed earlier, highlighting how small, simple molecules have often been a rich source of drugs historically.[82] However, given the challenging nature of many modern drug targets, it may be unrealistic to expect this proportion to be maintained for drugs in the future. It can also be seen from an examination of the fragment−protein complexes in the table and consideration of their respective affinities that it is not easy to predict with any confidence the potency of a fragment by considering how it is interacting with its target. This is also true of drug-sized ligands where nanomolar or micromolar compounds often look very similar in their binding modes within any given target protein. Potency (and therefore LE) is not simply the sum of the number of interactions and hydrophobic contacts a molecule makes; it is far more complex than that. This is why it is so hard to rank molecules in silico by virtual screening with any confidence and particularly difficult for fragments. What we can conclude, though, is that the maximal LE achievable will be a function of the binding site (not the fragment) and that for many targets very high LE has been observed historically, allowing easy detection of fragment binding and subsequent progression to low MWT drugs. Entry 7 in Table 1 illustrates this very clearly (PNMT). Here, the fragment-sized compound has extraordinary potency and LE ($IC_{50} = 0.003\ \mu M$, LE = 0.97) but has only a single polar interaction with the protein and quite limited van der Waals contact. The high LE observed is as much a function of the binding site as the fragment itself. This type of reasoning has led Hajduk et al. to propose that fragment screening can be used as an approach to examine target druggability, whereby if no hits are observed from FBDD, then the target can be considered intractable to small molecules.[127] The recent advent of FBDD is then simply a broadening of the use of fragments to targets that have a more typical maximal LE rather than limiting the use of fragments to highly tractable targets. For example, if we compare entries 10 and 11, we can see the fragments bind through a similar network of hydrogen bonds and they are of similar size. In the first case (tRNA-guanine transglycosylase) the compound has a potency of $IC_{50} = 2.1\ \mu M$ (LE = 0.65), while in the latter system (BACE-1) the fragment has $IC_{50} \approx 2000\ \mu M$ (LE ≈ 0.33). In the first example the compound was not considered to be a fragment, but instead a lead molecule, because of its high potency. In the latter case, FBDD methods were required for hit finding (i.e., a highly sensitive detection system (in this case X-ray crystallography)) and careful subsequent optimization was needed using SBDD approaches to convert the fragment into a lead series.[126] We can also see from examination of the fragment binding modes in the two proteins (entries 10 and 11) that we might anticipate that both of the fragments could bind to each of these proteins. This binding promiscuity is desirable within a fragment library and relates back to the sampling of chemical diversity discussed in section 2.5. Examination of the elaborated inhibitors in parts v and vi of Figure 4 together with a consideration of the shape of each of the active sites suggests that the larger lead compound would not be cross-reactive, illustrating how binding information will often be lost as MWT and chemical complexity increase.

Finally, in considering each of the eight examples where more potent lead molecules (Figure 4, section 3.2.2, and section 3.6.1) have been developed from the fragments pictured in Table 1, we see that the whole fragment or in one case (Figure 4i) the key region of the molecule maintains binding to the "hot spot"

Table 2. Clinical and Preclinical Candidates Derived from Fragments

compd	company	target	progress
LY-517717[a]	Lilly/Protherics	FXa	phase 2
PLX-204[b]	Plexxikon	PPAR agonist	phase 2
ABT-263[c]	Abbott	Bcl-X_L	phase 1/2a
AT9283[b]	Astex	Aurora	phase 1/2a
ABT-518[d]	Abbott	MMP-2 and 9	phase 1
AT7519[b]	Astex	CDKs	phase 1
PLX-4032[b]	Plexxikon	B-Raf^{V600E}	phase 1
SGX523[b]	SGX Pharmaceuticals	MET	phase 1
SNS-314[b]	Sunesis	Aurora	phase 1
NVP-AUY922[e]	Vernalis/Novartis	HSP90	phase 1
AT9311/LCQ195[b]	Astex/Novartis	CDKs	preclinical
AT13148[b]	Astex	PKB/Akt	preclinical
AT13387[b]	Astex	HSP90	preclinical
PLX-4720[f]	Plexxikon	B-Raf^{V600E}	preclinical
RO6266[g]	Roche	P38	preclinical
SGX393[b]	SGX Pharmaceuticals	BCR-Abl^{T315I}	preclinical

[a] *N*-[(1*R*)-2-[4-(1-Methyl-4-piperidinyl)-1-piperazinyl]-2-oxo-1-phenylethyl]-1*H*-indole-6-carboxamide. [b] Structure not disclosed. [c] Scheme 4, **11**. [d] (1*S*)-1-((4*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)-2-[[4-[4′-(trifluoromethoxy)-phenoxy]phenyl]sulfonyl]ethyl(hydroxy)formamide. [e] 5-(2,4-Dihydroxy-5-isopropylphenyl)-4-(4-morpholin-4-ylmethylphenyl)isoxazole-3-carboxylic acid ethylamide. [f] Propane-1-sulfonic acid [3-(5-chloro-1*H*-pyrrolo-[2,3-*b*]pyridine-3-carbonyl)-2,4-difluorophenyl]amide. [g] [6-(2,4-Difluorophenoxy)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]-((*R*)-2-methanesulfonyl-1-methylethyl)amine.

of each active site. In addition, in many of the examples described in section 3, there are structural data that support that the starting fragment has been evolved to a lead compound in which the core derived from the fragment recapitulates the interactions seen in original hit. Along with our own observations at Astex, this further supports that fragment evolution is generally a reliable process, despite the observation that fragment deconstruction may not always be so.[99]

FBDD cannot have truly been said to have come of age until a drug is launched that is derived from a low potency fragment. Table 2 lists the clinical and preclinical candidates and programs for which there is a clear statement in the public domain that the candidate drug has been derived using fragment-based drug discovery. In addition, Hajduk and Greer give a more comprehensive table of lead molecules derived from fragments.[16] Perhaps we would not expect a drug to have been born out of FBDD quite yet, as it took many years for SBDD to claim its first successful drug discovery projects.[128] However, these data support that the screening and optimization of fragments are beginning to have an impact on the clinical pipeline of companies. Of particular note in Table 2 is the discovery of **11** targeting the protein−protein interaction Bcl-X_L (section 3.2.1) currently in phase 1/2a clinical trials for cancer. This is one of the first non-natural product clinical agents developed against any protein−protein interaction drug target, and in a recent review on the topic, Wells and McClendon suggested that fragment screening may be a more fruitful approach than HTS for this extremely challenging area.[102]

So what does the future hold? One thing is certain. FBDD will not be a panacea that will solve all of our problems. Like structure-based drug design, computational chemistry, HTS, and combinatorial chemistry before it, FBDD will find an appropriate place in the armory of tools pharmaceutical companies employ to prosecute new targets. More than the underlying technology itself, the legacy of FBDD will be conceptual and that "less is more"; breaking down the problem of finding a drug molecule into its smallest component parts opens up new opportunities to solve challenging problems. The next chapter in the development of the field is likely to be the evaluation of FBDD against targets where high resolution structural information is more difficult to obtain. Only in this way can these key concepts be applied to the whole gamut of drug targets that the industry wants to work on. There are already companies committed to this approach. AstraZeneca have developed a work flow to tackle G-protein-coupled receptors and routinely screens a 20 000 membered fragment library against all its targets, independent of the likelihood of obtaining protein−ligand structures.[129] Ultimately, however, for success where experimental structural data are limited, methodology will need to be greatly improved to allow detailed SAR information to be generated in the 100 μM to 1 mM potency range so that empirical medicinal chemistry optimization becomes possible for fragments. This, coupled with continued breakthroughs in structural biology, particularly improving our understanding of the active sites of receptors (rather than enzymes) and further developments in modeling and computational chemistry, may soon make starting a drug discovery project from a fragment part of standard medicinal chemistry best practice rather than the specialized technology it once was.[130]

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Andrew J. Woodhead is a Senior Chemist at Astex Therapeutics. He has been with Astex since 2001 and has experience in fragment hit identification and structure based drug design for a number of targets including the oncology targets CDK2 and HSP90. He began his medicinal chemistry career with Wyeth in 1992, working on CNS disorders such as migraine and depression. In 1994 he joined Roche to focus initially on inflammatory diseases and then moved to the virology department before finally joining

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