Fragment-based lead discovery: leads by design

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Fragment-based lead discovery (also referred to as needles, shapes, binding elements, seed templates or scaffolds) is a new lead discovery approach in which much lower molecular weight (120–250Da) compounds are screened relative to HTS campaigns. Fragment-based hits are typically weak inhibitors (10μM–mM), and therefore need to be screened at higher concentration using very sensitive biophysical detection techniques such as protein crystallography and NMR as the primary screening techniques, rather than bioassays. Compared with HTS hits, these fragments are simpler, less functionalized compounds with correspondingly lower affinity. However, fragment hits typically possess high 'ligand efficiency' (binding affinity per heavy atom) and so are highly suitable for optimization into clinical candidates with good drug-like properties.

The problems with drug discovery productivity during the 1990s catalyzed significant investment by the pharmaceutical industry into major new high-throughput technologies such, as combinatorial chemistry and HTS. These techniques are now widely adopted and are central to most organizations' lead-generation approaches. HTS typically involves screening approximately a million relatively complex drug-sized compounds, with identification of the most potent hits as the primary objective. Combinatorial chemistry has been central to increasing the numbers of such compounds available for screening. Although these approaches have undoubtedly identified many high-value hits, the limitations of screening drug-sized compounds are starting to become apparent. Hit rates are often low and many of the hits fail to progress into optimization [1,2]. For those that do progress, their optimization into potent compounds tends to actually reduce their initial drug likeness and therefore reduce the developability of the final optimized compounds [3–5]. Fragment-based discovery [6,7] is targeted at addressing the issue of hit rate and also the ability to optimize hits into compounds possessing drug-like physical properties. The term 'fragment' is used here to describe a low molecular weight compound (~120–250Da) that is suitable for screening at high concentrations.

The hit rate from screening fragments is typically much higher than observed with HTS, as there is an inverse relationship between the molecular complexity of compounds screened and the probability of a compound possessing good complimentarity with the target protein [3]. Additionally, a library of small fragments represents a much higher proportion of the available 'chemical space' for low molecular weight compounds than a large library of drug-sized molecules does for higher molecular weight compounds, because the number of possible molecules rises exponentially as molecular weight increases [8]. A consequence of the higher hit rate for fragments is that fewer compounds need to be screened (typically <1000) to identify multiple hits, enabling fragment-based lead discovery to exploit a range of high information content screening techniques such as

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TABLE 1

Comparison of methods used for fragment screening arranged in order of decreasing throughput and increasing information content

Approach	Typical throughput per screen (compounds)	Quality of information about ligand binding mode	Resource and instrumentation requirements	Protein structure required	Key technical considerations	Representative references
HTS	100–1000K	None	Specialised infrastructure required	No	Not suitable for fragments	
High concentration bioassay	10–50K	None	Very straightforward method	No	High false-positive rates can often hinder interpretation of data	[17,20,31]
Surface plasmon resonance	10–50K	None	Straightforward method, but requires costly instrumentation	No	Protein or compounds must be immobilized; false positives possible	[32]
Affinity mass spectrometry	10–50K	None	Straightforward method, but requires costly instrumentation	No	Limited applications reported	[33,34]
Covalent attachment and MS	10–50K	None	Specialized infrastructure required	No	Requires cysteine residue close to active site	[22,35]
Dynamic combinatorial chemistry and LC/MS	1–10K	None	Straightforward method	No	Limited range of chemistry is suitable	[23,24]
Ligand-detected NMR (1D/2D)	1–10K	Can distinguish active site vs. non-active site binders	Straightforward methods using ^{1}H or ^{19}F, but requires costly instrumentation. Well-suited to screening of mixtures	No	Protein typically >20kDa in size; moderate protein requirements	[15,36,37]
Protein-detected NMR (2D)	1–10K	Information on principle interactions between ligand and protein	Requirement for labeled protein and (usually) $^{1}H/^{15}N$ NMR resonance assignments for amide groups. Requires costly instrumentation	Usually	Protein typically <30kDa in size; high protein requirements	[21,38]
X-ray crystallography	500–1000	Detailed binding mode elucidated	Specialized infrastructure required	Yes	Limited to ~35% drug targets where structure can be solved; moderate protein requirements	[18,39]

X-ray crystallography, NMR and biophysical methods, as well as bioassay techniques. Fragment-screening approaches need to possess high sensitivity in order to detect low-affinity hits in the millimolar or high micromolar range. In this context it is worth noting that, when some types of compounds are screened at high concentration (20–400μM), aggregation can occur leading to non-selective inhibition of many enzymes. This has been referred to as promiscuous aggregating inhibition [9] but such 'false positives' are more easily identified and are therefore much less problematic when biophysical techniques are used for the screening instead of conventional bioassays. Table 1 outlines the principle methods used for screening of fragments, compared with HTS.

Integration of protein X-ray crystallography into the subsequent screening cascade allows detailed structural ligand-binding information to be obtained and enables highly efficient hit validation and optimization. Figure 1 shows the differences between a typical HTS hit and a fragment hit and outlines the reasons why fragment optimization is often more straightforward.

Ligand efficiency and chemical tractability

The concept of ligand efficiency can be used to assess the quality of initial screening hits and also to monitor the quality of leads as they are being optimized. Hopkins *et al.* [10] have defined ligand efficiency (LE) simply as:

$$LE = -\Delta G/HAC \approx -RT\ln(IC_{50})/HAC \quad \text{[Equation 1]}$$

where ΔG is the free energy of binding of the ligand for a specific protein, HAC is the number of heavy atoms in the ligand and the IC_{50} represents the measured potency of the ligand for the protein. The origin of this concept can be traced back to pioneering work of Kuntz, Kollman and colleagues, who showed that, for strong-binding ligands of up to 15 non-hydrogen atoms, the free energy of binding is approximately 1.5 kcal/mol for each non-hydrogen atom [11].

Figure 2 illustrates the concept of ligand efficiency and how it relates to the 'chemical tractability' of a hit. The figure shows graphically a broad generalization of the range of molecular weights and potencies for HTS hits and fragments, superimposed on typical requirements for

leads using the same criteria. Fragment hits will be in the range 120–250Da and of low potency (mM to 30μM); to become useful leads, fragment potency will need to be increased, almost always with a resulting increase in molecular weight. HTS hits will have a much broader range of molecular weight (perhaps 250–600) and tend to be in the low μM to high nM potency range. Often molecular weight will need to be reduced and potency retained or increased to produce a quality lead series and experience shows that this is difficult to do. High quality lead molecules themselves tend to be relatively potent for their size, for instance, 100nM potency for 350Da.

The physical chemical properties of fragment libraries have received attention. For example, in order for fragments to be screened at high concentrations they require high solubility. Recent publications on NMR and X-ray-based screening against a range of targets indicate that the fragment hits obeyed, on average, a 'rule of 3', in which molecular weight is <300, hydrogen bond donors (HBD) ≤3, hydrogen bond acceptors (HBA) ≤3 and ClogP ≤3 [12]. These results imply that a 'rule of 3' could be useful for constructing fragment libraries for efficient lead discovery and distinguishes fragments from the larger, 'rule of 5' properties for drug-like compounds [13]. Some reports of approaches to the design of fragment-libraries have started to emerge [14–16].

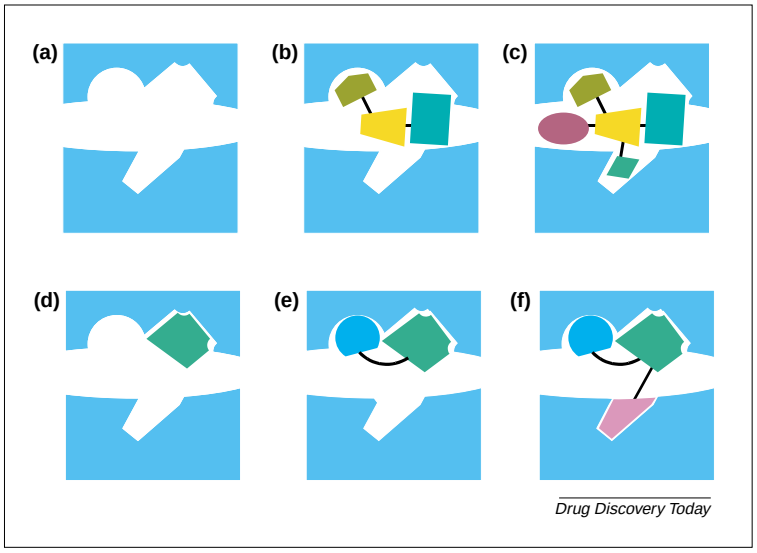

FIGURE 1

Schematic representation of 'drug-like' HTS hits and fragments as start points for drug discovery. (a) Cartoon representation of the active site of a protein, in which there are three pockets that are likely to be 'hot spots' for inhibitors to bind. **(b)** Representation of a typical drug-sized HTS hit binding to the active site. The HTS hit is functionally complex and makes numerous but low-quality interactions around the key pockets. The affinity is the summation of interactions spread across the whole molecule. **(c)** Representation of a potency-optimized compound derived from the HTS hit in **(b)**, which now better fills the binding site, but at the expense of increased molecular weight and significant complexity. Such optimized ligands are likely to have poor drug-like properties (e.g. size, synthetic complexity, low solubility, multiple functional groups that might be metabolized). **(d)** Representation of a 'ligand efficient' fragment (see main text) making a small number of high-efficiency interactions to one of the 'hot spots' within the active site. Due to their small size, such fragments would usually not have good activity in a biological assay (typically in the mM or high μM range). **(e)** Representation of ligand efficient hit compound making good quality interactions in the active site based around a small 'template'. Such a compound might be expected to be active in a biological assay. This lead-like compound might be rapidly identified from the fragment hit in **(d)** using structural information of how the fragment binds to the receptor. **(f)** Representation of an advanced lead, derived from the hit in **(e)**, making further high-efficiency interactions in the active site, while retaining the key interactions from the original fragment in the 'hot spot' of the binding site. This lead has been 'evolved' into neighbouring binding pockets to produce a compact, ligand efficient and potent lead. In general, X-ray crystallography of fragments binding to proteins indicates that fragments maintain the same interactions as those commonly associated with the final leads [16].

Examples of fragment based lead discovery

Examples of fragment based lead discovery have now been published on >26 protein targets [6,7] and a number of these examples are illustrated in Figure 3. The figure gives the lead compound identified after optimization of the fragment, and the fragment or fragments themselves are highlighted in each example. Examples a–d [17–20] illustrate the most frequently used approach, which is to identify initial fragments and then evolve them in an iterative manner using structure-guided design. Example e highlights an alternative but less commonly used approach, in which two fragments that bind proximally are linked using structure-based drug design [21]. Example f uses a more specialized technique, extended tethering, in which fragment space is explored via reversible covalent attachment to an active site residue [22], whereas in examples g and h, *in situ* coupling of fragments is performed under self-assembly conditions (dynamic combinatorial chemistry approaches) [23–25].

Example a

The lead was derived using a method referred to as 'needle screening' to identify compounds which bind to the ATP site of the bacterial enzyme DNA gyrase [17]. HTS screening against the target failed to identify any useful hits. Over a dozen classes of needle hits (or fragments) were identified using a high concentration bioassay and validated by biophysical methods including NMR, SPR and X-ray crystallography. One of the hits was indazole, which is highlighted in red in Figure 3. 3-D structural information of selected needles binding to the ATP-binding site was then used to guide optimization and gave >10,000-fold increase in activity relative to the starting indazole fragment (minimum non effective concentration (MNEC) in DNA gyrase inhibition 0.03 and >250μg/mL, respectively). The authors suggest that needle screening provides chemical starting points that have no unnecessary structural elements and hence reducing the risk of toxicity or metabolic instability.

Example b

A lead was discovered by use of high-throughput X-ray crystallography as the primary fragment screening technique [18]. A library of 'drug fragments' was soaked into crystals of P38α MAP kinase and hits such as the pyridine derivative, IC_{50}=1mM, were identified. Development of the fragment into drug-sized leads was then based upon

the X-ray structure of the fragment hits and led to the lead illustrated in Figure 3 (IC_{50}=100nM) with <70 compounds being synthesized in total.

Example c

The example illustrates the identification of a lead [19] from a fragment-based discovery programme that ultimately led to a clinical candidate. The known binder, benzamidine (200μM), was used as a 'seed template' for the S1 pocket of the blood coagulation enzyme Factor Xa. A structure-based virtual screening method was used to target proximal enzyme pockets and to drive three iterations of chemical synthesis. This identified compounds, such as the one shown with IC_{50}<20nM (>10,000 times more potent than the initial fragment). Subsequent optimization using a combination of medicinal chemistry and structure-based drug design led to replacement of the benzamidine, a moiety that is often associated with poor oral bioavailability. Further lead optimization, resulted in the discovery of the orally bioavailable candidate, LY517717.

Example d

This example uses a scaffold-based approach and screened a 20,000-member library via a bioassay for Phosphodiesterase IV inhibition [20]. Typically, scaffold-based libraries are slightly higher molecular weight and correspondingly larger in size than fragments. 316 compounds were identified as having >30% inhibition at 100μM and co-crystallization was employed to identify the binding modes. Structure-guided iterative library design was used to optimize the hits into potent leads with a 4,000-fold increase in potency.

Example e

The pioneering work of 'SAR-by-NMR' is illustrated by the identification of a potent 15nM inhibitor of the matrix metalloproteinase Stromelysin by linking two weaker inhibitors (17mM and 280μM) [26]. NMR screening of a set of a fragments in the presence of acetohydroxamic acid, which binds to the catalytic site zinc ion, allowed identification of the bi-aryl phenol inhibitor, binding in the P1' site of the enzyme. An NMR structure-determination of the two fragments bound in the active site of the protein allowed structure-guided design of the linked inhibitor. The SAR-by-NMR technique was not only one of the first [21] to be applied to fragment screening it is also one of the most widely used. A particularly powerful combination is the integration of NMR together with X-ray crystallography (for example, see Ref. [27]).

Example f

A technology termed extended tethering was used to identify a novel non-peptide inhibitor of caspase 3, a cysteine protease [22]. Mass spectrometry (MS) was used to identify the library members that form disulphide bonds to a tethered thiol present in the protein. Subsequently, these library fragments were coupled to known reversible cysteine-binding elements to generate potent, reversible molecules.

Example g

Self assembly of ligands can be catalyzed by binding of the building blocks of the reaction to the active site of proteins. A dynamic combinatorial library (DCL) has been used to identify a neuraminidase inhibitor from diamine (in red) and ketone building blocks [23,24]. The chemical formation of these inhibitor ligands is assisted by the presence of the neuraminidase protein. The structure of the lead shown in Figure 3 (Ki=85nM) is closely related to the active component of oseltamivir, a marketed influenza neuraminidase inhibitor.

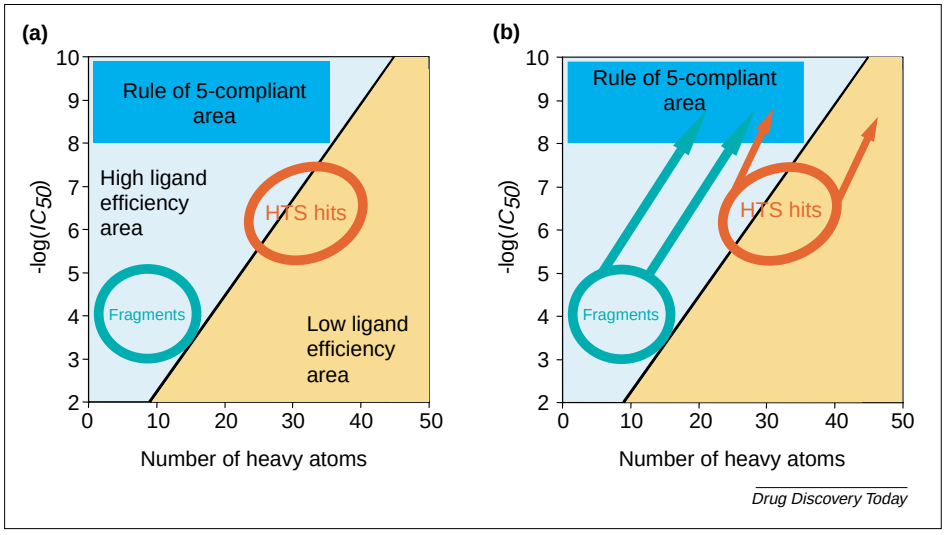

FIGURE 2

Ligand efficiency and chemical tractability of a hit. (a) The concept of ligand efficiency (LE) can be used to assess the quality of initial screening hits and also to monitor the quality of the leads as they are optimized [10]. To obtain a 10nM optimized compound that fits Lipinski's molecular weight guide for oral activity (i.e. molecular weight <500) [13] then the maximum number of heavy atoms the compound can contain is ~36, leading to an LE of around 0.3. LE=0.3 can then be viewed as the minimum ligand efficiency and is the line separating the blue and orange areas on graph **(a)**. The darker blue area is the region where compounds would be at least 10nM in potency and also obey Lipinski's molecular weight guide. Low-affinity and/or low-molecular weight fragments are shown in the green circle, and it can be seen that such compounds fall in the high ligand efficiency area. Fragments of low ligand efficiency will not generally be detectable or will not bind to the protein target at all. The red oval depicts the broad cross section of assay-detectable hits from HTS, and includes low molecular weight lead-like compounds that would be seen as chemically tractable, as well as many more less-attractive compounds with poor ligand efficiency. **(b)** Potency optimization of fragments or HTS hits will tend to be linked to an increase in complexity and molecular weight [3–5]. An efficient optimization will be one in which potency is increased without a reduction in ligand efficiency. This is illustrated by the green and red arrows from the fragments and HTS regions respectively, running parallel to the line of constant ligand efficiency. Due to the low complexity and molecular weight of fragments, an optimization that maintains ligand efficiency should result in a rule of 5-compliant lead compound. However, optimization of an HTS hit with poor initial ligand efficiency, will not in general result in a rule of 5-compliant compound, but instead will give a potent, high-molecular weight ligand that is unlikely to be useful as a drug candidate. Lead-like ligand-efficient HTS hits can usually be optimized to rule of 5-compliant compounds. Of course, there are many exceptions to these generalizations.

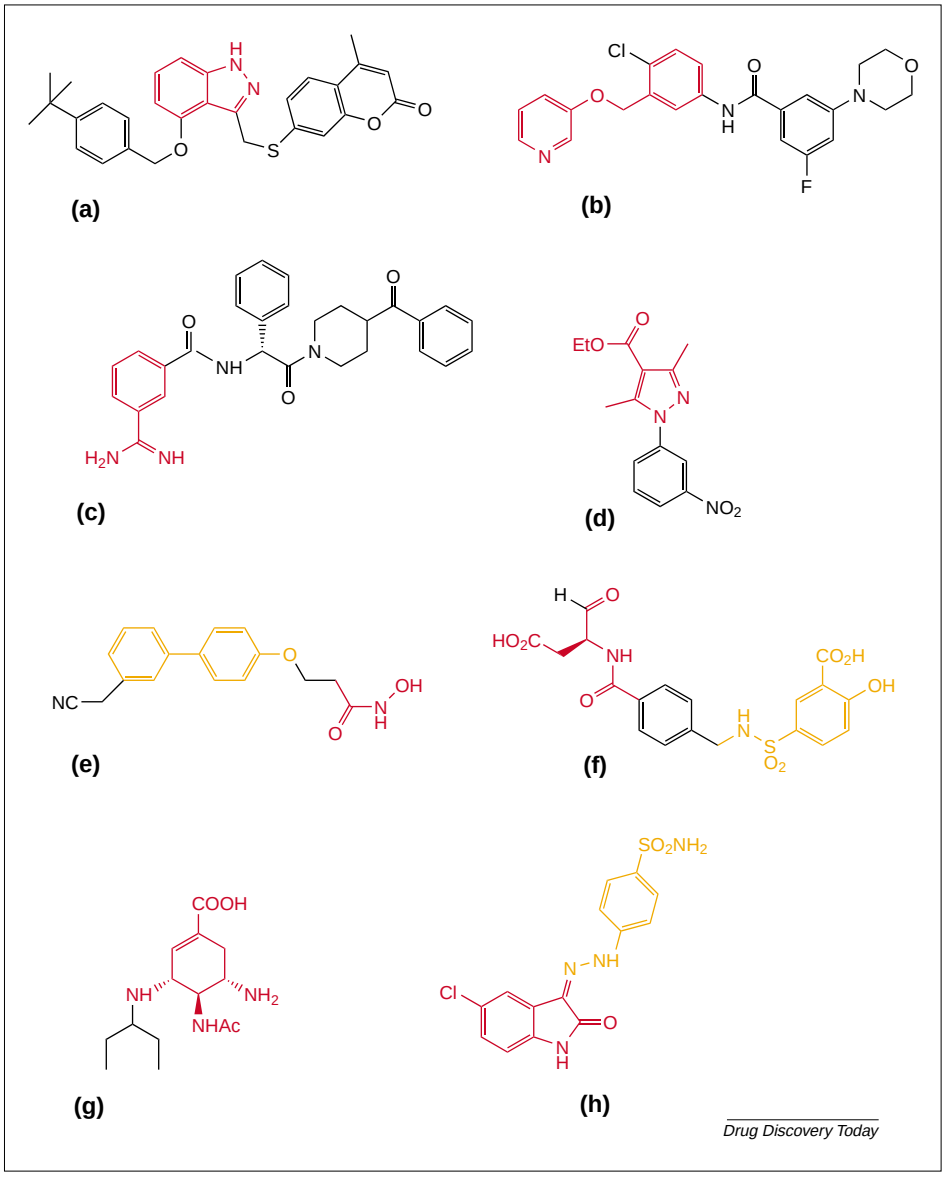

FIGURE 3

Examples of lead compounds derived using fragment-based methods with the original starting fragments shown in red and orange. Where available the potencies of the starting fragment and the final molecule are given for each case together with the calculated ligand efficiencies in parentheses. **(a)** DNA gyrase: Kd = 10mM (by NMR) → MNEC = 30ng/ml. **(b)** P38 kinase: IC50 ~ 1mM (LE=0.29) → IC_{50} = 65nM (LE=0.32). **(c)** Factor Xa: Ki = 200μM (LE=0.56) → Ki = 16nM (LE=0.30). **(d)** PDEIV: IC_{50} = 60μM (LE=0.48) → IC_{50}=33nM (LE=0.49). **(e)** Stromelysin: Kd = 17mM (LE=0.48) (red) and Kd = 280•M (LE=0.37) (orange) → Kd = 15nM (LE=0.49). **(f)** Caspase: extended tethering → Ki app = 200nM. **(g)** Neuraminidase: dynamic combinatorial library → Ki = 85nM (LE=0.48). **(h)** Cdk2: DCXTM → IC_{50} = 30nM (LE=0.45)

Ligand efficiency of examples

Where a K_i, K_d or IC_{50} was available, a calculated ligand efficiency (LE) has been given in Figure 3 for the lead compound and the starting fragment(s). It can be seen that the LEs of the lead compounds are all 0.3 or better, indicating that these leads are 'on track' to deliver clinical candidates that obey the Lipinski molecular weight guide. Additionally, in the majority of cases (examples d, e, g and h), the lead compounds have efficiencies between 0.45–0.49 and so have the potential to yield candidates in the molecular weight range 300–340Da, close to the mean molecular weight of 337Da for marketed oral drugs [28]. Examples b and c give respectable LEs (~0.3) and examination of the original references reveals that in the case of Factor Xa [19], the LE is similar to the cited competitor compound, DX-9065a (LE=0.31), whereas for p38 [18] the cited compound, SB203580, also has similar efficiency (LE=0.33). In these cases, the fragment-based approach has therefore been able to produce compounds of similar efficiency to alternative technologies.

Examination of the fragment efficiencies in Figure 3 shows that it is often possible to maintain LE during the fragment optimization process – at least for cases where structure-based drug design can be used extensively. It is clearly important to start the optimization from as efficient a fragment as possible, and the use of fragments that are well represented in drugs prevents the introduction of non-drug-like moieties that form strong interactions. Wermuth has given multiple examples where drug molecules themselves have been used as leads to design selective compounds for alternative indications [29], and so cross-reactivity of fragments between different target classes should not, in general, represent an obstacle to lead identification and optimization.

Example h

A complimentary approach has been reported in which inhibitors are formed from a DCL in the presence of protein crystals. The binary complex is then observed directly by X-ray crystallography [25]. This has been termed 'dynamic combinatorial X-ray crystallography' (DCX™) and is illustrated by the identification of a previously reported inhibitor of cyclin dependent kinase-2 (IC_{50} = 30nM) from a mixture of essentially inactive hydrazines (in orange) and isatins (in red) as potential fragments for adjacent binding pockets within the ATP-site.

Summary and outlook

With the first worked examples appearing several years ago, and a significant number of additional papers having now published, fragment-based discovery is currently at a transitional stage in its development. The concepts behind the approach including decreasing the numbers but increasing the information content during lead discovery appear to have gained broad acceptance. Attention is now focused on deciding when and how best to use fragment-based discovery so that it adds significant value

to lead and drug discovery programmes. The majority of examples of fragment-based discovery in the literature are for targets where NMR and/or X-ray can obtain structural information. Such structural information improves the efficiency of hit validation and hit progression significantly. Although fragment-based discovery can, in principle, be applied to targets and classes of targets where such structural information is not available, the progression of such very weak hits without structural information to guide validation and optimization is a much less attractive option.

Typically, new technologies offer two potential benefits: to enable you to do something that you could not previously do, and to enable you to do something more efficiently than you were previously able to do it. There are now increasing numbers of examples appearing in the literature that demonstrate that fragment-based discovery can identify quality leads for targets where HTS has not succeeded [6,7,30]. The second benefit, establishing that a fragment-based approach increases drug discovery efficiency, will by necessity take longer to establish. It can be argued that published fragment-based leads with high ligand efficiency and good lead-like physical properties are higher quality leads than most HTS derived leads, but ultimately this is a subjective judgment, and probably the best assessment of the quality of a lead is the ability to progress it efficiently into a clinically successful compound. Although we have already seen the first clinically successful compound from this approach, further such successes over the next few years will be required before the full potential of this new lead discovery approach can be established.

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