



# From fragment to clinical candidate—a historical perspective

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As recently as ten years ago few scientists had heard of fragment screening, let alone considered low molecular weight fragments (MW <300) with weak binding affinities to be attractive start points for drug discovery programmes. Today, however, there is widespread acceptance that these fragments can be progressed into lead series and on to become clinical candidates. Consequently, over the past three to four years, fragment-based drug discovery has become firmly established within the biotechnology and pharmaceutical industries as a complimentary strategy to high-throughput screening. In this review, we give a historical perspective of how rapidly fragment-based drug discovery has developed and describe a number of clinical compounds discovered using this approach.

The ability to identify small molecules that interact with a biological target is a key step in drug discovery. To date, we are not able to quantitatively predict interactions between biological systems and small molecules, instead screening programmes have to be employed. The most successful approaches have been identification of leads from natural products, fast follower programmes or through the random screening of compound collections [1–4].

Over the past few decades, the pharmaceutical industry has invested heavily in technologies such as combinatorial chemistry and high-throughput screening (HTS) resulting in the rapid screening of large compound libraries [5]. Historically, HTS libraries have been populated with ‘drug-like’ molecules that have been selected to broadly comply with Lipinski’s rule of five [6]. HTS has undoubtedly produced high quality lead molecules for many drug discovery programmes; however, hit rates are generally low, particularly when screening against challenging targets [7,8]. Additionally, optimisation of lead compounds can be problematic owing to the often large and relatively lipophilic nature of the screening hits [9]. The past 10 years has seen the screening of fragments evolve from a niche area of research to become an important alternative to HTS. Fragments are defined as low molecular weight (MW <300), moderately lipophilic (clogP < 3), highly soluble organic molecules, which typically

bind to their target protein with low affinity (in the  $\mu\text{M}$  to mM range) [10]. In order to detect these weak binding events, sensitive biophysical techniques such as NMR [11–14] or X-ray crystallography [15–17] need to be employed for screening. In general, fragment screening (FS) delivers higher hit rates than HTS, mainly due to the fact that large compounds have a greater chance of a mismatch with the target protein binding site [18]. Additionally, since the size of chemical space increases exponentially with molecular size, statistically, covering chemical space is more manageable with fragments than with drug-sized molecules [19,20]. To date, FS has been used both as a primary screening tool and in instances when a HTS campaign has failed to identify useful starting points. It is now becoming common practice, particularly within large pharmaceutical companies to run FS in parallel with HTS.

In this review we will provide an historical view on how the seminal concepts and ideas around fragments binding have evolved and been applied to drug design. Figure 1 highlights some of the major contributions to date, from the first literature mention of fragment binding over 25 years ago, to some of the major advances in FS and finally on to the first fragment derived compounds entering clinical trials. These advances have allowed FBDD to become the widely accepted technique it is today. It should be noted, however, that this is by no means an exhaustive list and readers are directed to other reviews that have been published in recent years [21–24].

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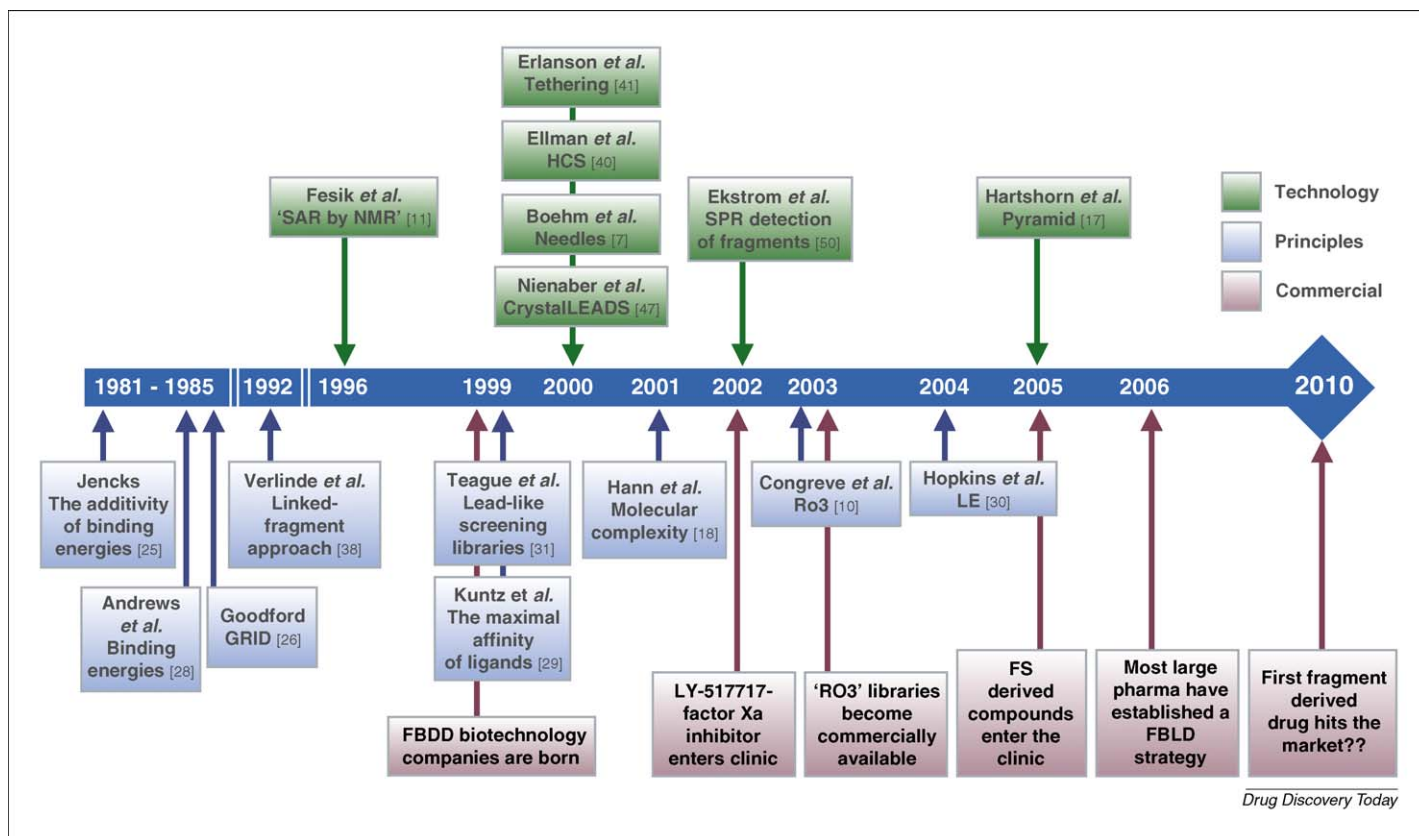


FIGURE 1

Timeline—selected landmarks that have significantly influenced the field of FBDD.

## Principles of fragment screening

### Fragment binding

The fundamental principles of molecular recognition, which have played a key role in the development of fragment-based drug discovery (FBDD) have been around for over two decades. In 1981, Jencks introduced the idea that large molecules can be considered as the combination of two or more 'fragments' that contain all the features necessary for binding to the target protein [25]. In 1985, Goodford developed a computer program called GRID where small fragments or 'probes' were used to scan a putative binding site in order to highlight interaction hot spots [26]. GRID analysis applied to a few protein binding sites indicated that some of the favourable positions found for individual molecular fragments were present in known inhibitors. Fragments and large molecules have to obey to the same set of molecular recognition rules when they bind to a target protein. During a binding event both fragments and large molecules have to overcome an entropic barrier associated with the loss of rigid body entropy on binding to the target. This barrier has only a small dependence on molecular size [27]. Fragments can afford the entropic cost of binding only by using a high proportion of their atoms to form interactions; therefore, fragments are considered efficient binders (high binding energies per unit of molecular mass).

Optimisation of a weak-binding fragment into a potent lead molecule can usually be achieved by adding extra atoms onto the fragment core. The maximal jump in affinity obtainable by adding an additional group to a fragment core can be estimated using a theoretical model developed in 1984 by Andrews *et al.* [28]. The maximal affinity of ligands was also investigated in 1999 by the

Kuntz group. Those studies indicated that potency could increase by up to 1.5 kcal/mol per heavy atom added to the original fragment [29]. However, when the free energy of binding reaches about 12 kcal/mol ( $K_d \sim 1$  nanomolar), these increments become much smaller. In 2006, Hajduk and co-workers revisited the concept of maximal affinity by using the data from 15 Abbott discovery programmes [23]. The results of this study confirmed those of Kuntz and co-workers for ligands consisting of 5–25 atoms. Hajduk observed that successful hit-to-lead optimisations steadily increase the free energy by 0.3 kcal/mol per additional atom, until the limit of 12 kcal/mol was reached. A value of 0.3 kcal/mole per heavy atom was also suggested by Hopkins and co-workers as a lower limit of Ligand Efficiency (LE) for fragment hit selection [30] (See Box 1 for a definition of LE). The work of the groups of Kuntz and Hajduk also highlights that large compounds with more than 20 heavy atoms tend to be less efficient binders than fragment sized compounds.

In 2001, Hann *et al.* used a simple model of ligand–receptor interactions to investigate the impact of molecular complexity on binding to a protein and on the probability of identifying hits or leads [18]. Hann's model suggested that large and complex molecules have a greater probability of forming suboptimal or repulsive interactions with the receptor. By contrast, fragments have a lower chance of forming mismatches and as a consequence, hit rates from FS are often higher and the hits identified are generally more ligand efficient.

### Fragment libraries

In 1999, Teague *et al.* used data from high-throughput screens at AstraZeneca to analyse and improve HTS libraries. Their study

**BOX 1**

FBDD—fragment-based drug discovery

FS—fragment screening

Ligand Efficiency—Ligand Efficiency (LE) is defined as the free energy of binding of a ligand for a specific target averaged for each heavy atom [30]. This value gives an indication of the quality of hit.

$$LE = -\Delta G/HAC \approx -RT \ln(IC_{50})/\text{number of non-hydrogen atoms}$$

LE is used to compare hits across series and to guide the optimisation of fragments into lead molecules. LE values greater than 0.3 kcal mol<sup>-1</sup> per non-hydrogen atom are desirable to obtain oral drug candidates, which also fulfil Lipinski's rules. LE can also be used to assess the druggability of a particular target. LE values above 0.4 are usually associated with deep and well defined binding pockets (e.g. kinases or GPCRs) whereas LE values around 0.3 and below are common in featureless and solvent exposed pockets (e.g. proteases or protein-protein interactions), which are less druggable. A number of alternative definitions of LE have been proposed [57–59] and there is also an increased interest on normalising potency by lipophilicity [60].

Low affinity fragment—a low molecular weight compound (MW typically <300) with a binding affinity in the μM to mM range.  
Fast follower programme—the design of novel biologically active compounds from competitor information, such as that found in the patent literature.

highlighted that polar low molecular weight hits were more easily elaborated into leads than higher molecular weight lipophilic hits [31]. These findings prompted a paradigm shift in what was perceived to be a good screening library. Previously, key factors in screening library design were library size, molecular diversity and drug-like physicochemical properties. Teague *et al.* proposed the use of libraries with a molecular weight range between 100 and 350 and clogP = 1–3, which would allow sufficient opportunity for further optimisation of potency and physicochemical properties of the identified leads. In essence, the properties of drug molecules are distinct from the properties of their original leads [32]. The following five years saw a marked increase in the number of publications discussing the make-up of screening libraries, from re-designing HTS libraries, to NMR screening libraries and general fragment libraries. The make-up of screening libraries has continued to evolve as more has been learned about

the strengths and weaknesses of various screening systems. When designing a FS library there are a number of important considerations. Owing to the limited size and complexity of fragments, they generally bind to a target protein with very low affinity and as such they are required to be screened at very high concentration, consequently fragments should have high aqueous solubility. To achieve this, the range of physicochemical parameters of the fragments should be carefully considered, including a molecular weight maximum of around 300 Da, cLogP < 3 and complexity filters such as limiting the number of H-bond donors, H-bond acceptors and rotatable bonds (as proposed originally in the 'rule of three' (Ro3<sup>TM</sup>)[10]). Other important factors to consider as with any screening library are molecular diversity, privileged medicinal chemistry scaffolds, drug-like precedent, novelty and synthetic tractability.

Compound suppliers were quick to respond to the increased demand for fragment and lead-like libraries. The first commercially available fragment libraries went onto the market in 2003, with Asinex and Maybridge supplying the first Ro3 compliant collections. A number of vendors currently sell commercial fragment libraries, many with the added benefit of access to large follow-up collections (see Table 1). Commercial fragment libraries particularly benefit small biotechnology companies and academic institutions as they are able to access compound collections without spending a significant amount of time and resource assembling one.

### Fragment screening methodology

FS has been carried out using many detection techniques, all of which are required to be run at high concentrations (in the μM to mM range) in order to detect weakly binding fragments. These methods include high concentration biochemical assays [33] and biophysical methods such as NMR [34], mass spectrometry [35], X-ray crystallography [36] and surface plasmon resonance (SPR) [37], or perhaps more commonly a combination of a number of these methods. One of the earliest reported attempts at screening fragments was in the early 1990s at the University of Groningen, where a method was developed to identify and link together low molecular weight molecules (fragments containing 4–11 heavy atoms) as 'building blocks', in order to develop lead compounds

**TABLE 1**

#### Chemical suppliers that provide fragment screening libraries

Supplier	Library name	Number of fragments
ACB Block	Fragment library	1280
ACB Block	F-Library for NMR	760
ASDI	Fragment screening collection	nd
Asinex	Building blocks	6200
BIONET	Fragment library	3228
Chembridge	Fragment library	3800
Enamine	Fragment library	11717
Maybridge	Br-fragment collection for X-ray	1500
Maybridge	F-fragment collection for NMR	5300
IOTA Pharmaceuticals	Fragment library	5500
Zenobia Therapeutics	Commercial fragment library	352

for the treatment of sleeping sickness [38]. The same group developed this methodology further by using X-ray crystallography as a tool to screen mixtures of compounds [39].

The late 1990s and early 2000s saw the publication of a number of important papers that have proven influential in shaping the way FBDD has developed.

In 1996, Fesik and co-workers from Abbott reported the use of 'SAR by NMR' [11]. This technique makes use of changes in the  $^{15}\text{N}$ -labelled protein NMR spectra upon ligand binding and was used to identify low affinity fragments binding in adjacent pockets of the FK506 binding protein. It proved possible using NMR derived structural information to link these fragments with a short flexible linker resulting in a low nM inhibitor.

The year 2000 saw a flood of interesting papers describing a range of strategies for screening fragments. A group from Roche introduced the concept of 'needle' screening with a 'needle' being defined as a low molecular weight ligand [7]. After an unsuccessful HTS campaign against DNA gyrase, the Roche group developed a method for screening low molecular weight inhibitors. Compounds were initially screened *in silico* using detailed 3D structural information, and then followed up by a HTS assay specifically developed for running at high concentration. Hits were confirmed using multiple biophysical techniques including SPR, NMR and X-ray crystallography, highlighting the benefit of using orthogonal detection methods for confirmation of fragment binding. In the same year Ellman and co-workers used combinatorial target guided ligand assembly to identify nM c-Src inhibitors. These two examples appear to be some of the earliest reports of FS using high concentration biochemical assays [40].

The year 2000 also saw the introduction of Sunesis's mass spectrometry detected 'tethering' technique. 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 [41]. The use of a protein target as a scaffold, allowing suitable fragments to bind, react and form more potent elaborated inhibitors has continued to expand with the advent of 'click chemistry' [42] and directed combinatorial X-ray crystallography [43].

X-ray crystallography has long been recognised as a valuable tool within the drug discovery community, its use in SBDD has been well documented and it is also well suited to FS [44–46]. In addition to developing NMR screening methodology, a group from Abbott published on the use of X-ray screening (Crystal-EAD) to identify low affinity hits against the anticancer target urokinase [47]. Soaking apo crystals of urokinase with solutions containing mixtures of fragments with high shape diversity, followed by X-ray analysis led to the detection of a number of hits. Subsequent structure based drug design (SBDD) led to the identification of an orally bioavailable compound with nM activity. Fragment screening by X-ray crystallography has continued to evolve, automation of crystal manipulation using robotic systems and the use of electron density interpretation software such as AutoSolve<sup>TM</sup> has dramatically reduced the time taken for co-crystal structure determination, making this process an effective screening technique [15,48]. In 2005, Hartshorn *et al.* published on fragment screening using high-throughput X-ray

crystallography to identify hits against a number of different protein classes including kinases, proteases and phosphatases [17].

Initially developed as bioanalytical tool, SPR has become an increasingly popular primary screening technique within drug discovery, able to generate both affinity data and kinetic information for biomolecular interactions. The technique relies on either the target or test compounds being immobilised on a sensor surface, then monitoring for changes in refractive index on test compound binding. In 2002, Ekstrom *et al.* used SPR to detect a range of fragment sized molecules binding to an allosteric purine binding site of human liver glycogen phosphorylase with affinities in the low  $\mu\text{M}$  to mM range. Confirmation of binding mode was carried out by X-ray crystal structure determination for three of the compounds, providing valuable insight into the SAR of this series of compounds and potential areas for future development [49].

Continued technological developments across all the major screening platforms have resulted in benefits such as enhanced sensitivity, higher throughput and applicability to more diverse biological targets. Readers are directed to the other reviews that describe these areas in more detail [21,22,50].

### From fragment to clinic

The late 1990s saw the emergence of a number of fragment-based biotechnology companies, including Astex (specialising in high-throughput X-ray crystallography), Sunesis (tethering, using mass spectrometry detection), SGX (X-ray crystallography and high concentration screening—HCS), Plexxikon (HCS followed by X-ray) and Evotec (HCS/NMR followed by X-ray crystallography). These companies, along with Abbott in particular from the pharmaceutical industry, have been instrumental in demonstrating that FBDD is a valid way of progressing compounds into clinical development.

Perhaps the first fragment derived compound to enter clinical trials was LY-517717 a factor Xa inhibitor discovered by Tularik/Protherics and Lilly in late 2002. Around 2005, compounds derived from FS approaches began to enter clinical trials, with AT7519 (a CDK2 inhibitor from Astex), ABT-737 (a Bcl-X<sub>L</sub> inhibitor from Abbott and the precursor to ABT-263) and PLX-204 (a PPAR agonist from Plexxikon) being some of the first (Table 2).

By 2006, perhaps partly in response to the success of early FBDD programmes and partly due to an industry wide rethink of the composition of screening collections, the majority of large pharmaceutical companies had begun to incorporate FS into their hit identification strategies. High concentration or 'reduced complexity' screening (HCS) is widely employed throughout the industry and allows the use of existing high-throughput screening platforms without the upfront expense of new technology and expertise. It will be interesting to hear over the coming years how FBDD is integrated alongside HTS within the pharma industry, and see many more examples of fragment to candidate optimisation.

In the following section, we discuss four examples where a low affinity fragment has been progressed into a clinical candidate. The key binding motif can be traced from fragment to advanced compound (Figure 2), although in a number of cases the initial fragment has been modified significantly to aid optimisation.



TABLE 2

## Fragment derived compounds and furthest stage of clinical development

Compound	Company	Target	Progress	Detection method
ABT-263	Abbott	Bcl-X <sub>L</sub>	Phase 2	NMR
AT9283	Astex	Aurora	Phase 2	X-ray
LY-517717	Lilly/Protherics	FXa	Phase 2	Computational/X-ray
NVP-AUY-922	Novartis/Vernalis	Hsp90	Phase 2	NMR
Indeglitazar	Plexxikon	PPAR agonist	Phase 2	HCS/X-ray
ABT-518	Abbott	MMP-2 & 9	Phase 1	NMR
AT7519	Astex	CDK2	Phase 1	X-ray
AT13387	Astex	Hsp90	Phase 1	NMR/X-ray
IC-776	Lilly/ICOS	LFA-1	Phase 1	NMR
PLX-4032	Plexxikon	B-Raf <sup>V600E</sup>	Phase 1	HCS/X-ray
PLX-5568	Plexxikon	Kinase Inhibitor	Phase 1	HCS/X-ray
SGX-523	SGX Pharmaceuticals	Met	Phase 1	X-ray/HCS
SNS-314	Sunesis	Aurora	Phase 1	MS

*Bcl-X<sub>L</sub>*

The NMR-based screening method 'SAR by NMR' has been used by Abbott to generate fragment hits against the cancer target Bcl-X<sub>L</sub>. Fragment screening was chosen after initial attempts at conventional HTS against Bcl-X<sub>L</sub> failed to yield progressable hits. 'SAR by NMR' identified two fragments **1** and **2** (Figure 2a) bound at the protein surface in two distinct subpockets. Structural information gleaned by NMR was used as a basis to link the two fragments, replacing the carboxylate with a linking acyl sulfonamide, which allowed the acidic nature to be maintained. Further elaboration gave compound **3** that has activity in the nM range. Significant optimisation efforts led to the discovery of **4** (ABT-263), an agent suitable for oral dosing, which is currently in phase II clinical trials for cancer. To our knowledge, ABT-263 is the most advanced clinical compound that has been derived from a FS approach [51].

*CDK2*

Researchers at Astex have used high-throughput X-ray crystallographic screening to identify a number of fragment hits binding to cyclin-dependent kinase 2 (CDK2), a potential target for cancer chemotherapy. Structure-guided optimisation of indazole **5** (Figure 2b) helped to identify key binding features for this class of compound and key areas to elaborate further. The indazole template was evolved into pyrazole **6** resulting in an additional protein–ligand H-bonding interaction to the hinge region of the kinase. X-ray crystallographic data was used to identify other key binding features including a water-mediated hydrogen bonding interaction that contributed to the identification of lead compound **7**. Further optimisation of selectivity, cellular activity and pharmacokinetic properties led to the discovery of diamide **8** (AT7519), which induces tumour regression in a number of cancer models and is currently being evaluated in clinical trials for the treatment of various cancers [52].

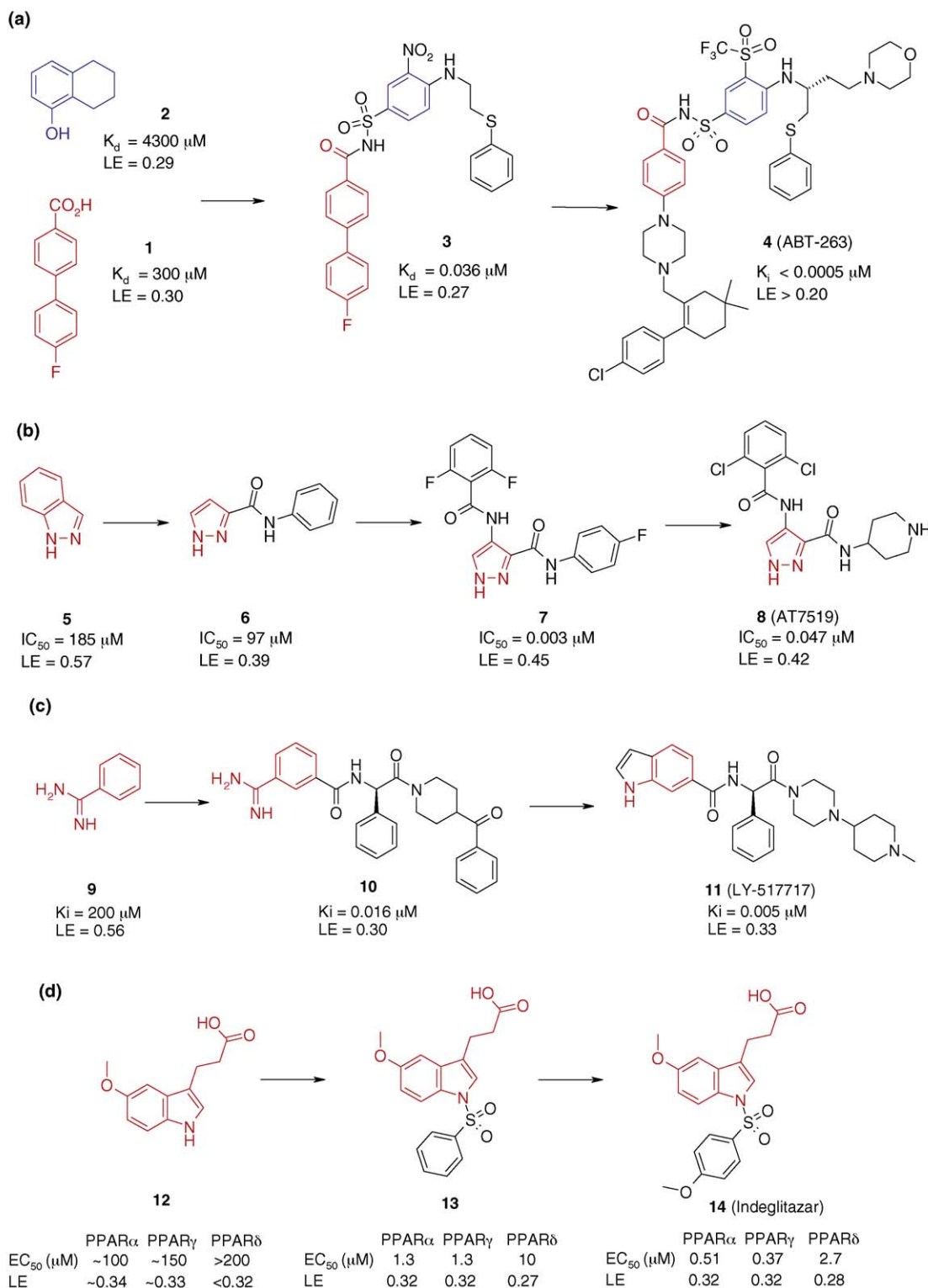
*Factor Xa*

Researchers at Protherics used library design and virtual screening to develop inhibitors of Factor Xa, an important target in the blood coagulation cascade. Starting from benzamidine **9** (Figure 2c), a

fragment with a well defined binding mode from X-ray crystallographic data, iterative library design identified a number of lead molecules such as amidine **10** [53]. Amidine containing compounds were shown to have anti-thrombotic activity but poor oral bioavailability, presumably owing to the presence of the highly basic benzamidine moiety, which is known to prevent absorption. The benzamidine was replaced with an uncharged indole, which can still form favourable interactions with the catalytic aspartate residue and gave rise to compound **11** (LY-517717). LY-517717 has shown proof of concept in a phase II clinical trial as an antithrombotic [54].

*PPAR*

High concentration screening of a low molecular weight library (150–350 D) was used by scientists at Plexxikon to identify hits against all three peroxisome proliferator activated receptors (PPARs), PPAR $\alpha$ , PPAR $\delta$  and PPAR $\gamma$  [55]. Modulation of PPAR activities is expected to be useful in the treatment of Type 2 diabetes mellitus. After the primary HCS screen, 170 potential hits were selected for co-crystallisation with at least one of the three receptors. Around 25% of the molecules produced a structure with at least one of the PPARs. The X-ray crystal structure of **12** (Figure 2d) co-complexed with PPAR $\gamma$  showed that the indole fragment had a unique binding mode when compared with other known PPAR ligands. The indole N-1 position was subsequently used as a growing point to probe an adjacent pocket, which was highlighted in a PPAR pan-activity field analysis as an energetically favourable region. SBDD led to the development of the low micromolar phenyl sulfonamide **13**. On the basis of this success, additional sulfonamide side chains were evaluated computationally and 20 sulfonamide analogues were selected for synthesis and subsequent testing in biochemical and cellular assays. From this small set of compounds, Indeglitazar **14** emerged as a full agonist of PPAR $\alpha$  and partial agonist of PPAR $\gamma$ . This particular profile was preferred in order to avoid the clinically observed side effects of full PPAR $\gamma$  agonists. Indeglitazar was progressed to phase II trials for Type 2 diabetes. Recently, the development of Indeglitazar was suspended owing to increasing concerns over the potential side effects of PPAR agonists.



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**FIGURE 2**

Four examples highlighting the progression of fragment hits to clinical candidates for (a) Bcl-X<sub>L</sub>, (b) CDK2, (c) FXa and (d) PPAR. The coloured moieties of the final compounds indicate the functionality derived from the original fragments.

As the examples highlighted in the previous sections attest to, there is no single correct way to carry out FBDD. Many companies have their own unique approach; however, it is apparent from many examples in the literature that structural data can greatly accelerate the hit to lead process. Our experience at Astex suggests that careful optimisation of binding affinity using structural data and a normalised potency coefficient (such as LE) has routinely provided small and potent lead compounds. However, fragment optimisation should not be guided by potency alone. The use of normalised physicochemical properties throughout the fragment evolution process provides useful guidelines for discovering high quality leads. As a consequence of this the lead optimisation phase is often greatly accelerated, resulting in clinical candidates with good drug-like properties (lower MW and clogP than the industry standard).

## Conclusion

Screening fragments, particularly when using sensitive biophysical techniques has a number of important benefits that should in the future allow this methodology to tackle some of the more challenging drug discovery targets. Fragment libraries statistically cover chemical space better than drug-like or lead-like libraries and as a consequence fewer compounds need to be screened. Also FS tends to deliver high hit rates with the additional benefit of providing multiple start points for optimisation programmes. Biophysical techniques, owing to their high sensitivity, can identify very weak

interactions. This is highlighted by the Bcl-X<sub>L</sub> example, where non-peptidic inhibitors of a protein–protein interaction (PPI) target have been identified. It is anticipated that FS will be an effective way of probing other PPIs by detecting very weak interactions, identifying new potentially transient binding pockets or allosteric binding sites. Progress has also been made into looking at membrane bound drug targets such as G-protein-coupled receptors (GPCRs). Recent publications on the production of stable, properly folded membrane protein may make this class of targets amenable to structural and biophysical characterisation and subsequently FS [56].

The future for this approach looks bright with groups from academia, the pharmaceutical and biotechnology sectors carrying out FBDD. As indicated in Table 2, a number of compounds have already progressed to phase I and II clinical trials with many more in pre-clinical development. It may still be a number of years before we see a fragment-based drug reach the marketplace; however, it does appear as though FBDD is beginning to have an impact on clinical pipelines in the pharmaceutical industry.

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