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FRAGMENT-BASED LEAD DISCOVERY

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ABSTRACT

Fragment-based drug discovery (FBDD) represents a logical and efficient approach to lead discovery and optimisation. It construct a drug leads from small molecular fragments and gaining momentum in both large pharmaceutical companies and biotechnology laboratories as a complementary approach to traditional screening. This is because fragment-based approaches require significantly fewer compounds to be screened and synthesized, and are showing a high success rate in generating chemical series with lead-like properties. In this it is a highly efficient method for drug discovery, and the techniques which are popular in the past few years. In this review, I describe how a variety of approaches in fragment-based lead discovery — by NMR, X-ray crystallography, mass spectrometry, functional screening, in silico screening have produced drug leads. The examples show that the technique can reliably generate potent molecules, there is still much work to be done to maintain the efficiency of molecules' binding affinities as fragments are linked, expanded, and otherwise improved.

Keywords: FBDD, Scaffold based drug design, Screening fragments, Fragment growing, Target proteins.

INTRODUCTION

Small-molecule drug discovery has always been a struggle of attrition, but in the past few years pressures have mounted to increase efficiency at all stages of the process.A potential solution for lead identification and optimization, fragment-based lead discovery, is becoming increasingly popular [1]. The goal is to build drug leads in pieces, by identifying small molecular fragments and then either linking them or expanding them. The definition of a fragment varies, but usually refers to molecules weighing less than 200–300 Dalton, with fewer than 15–20 heavy atoms. The concept of fragment-based lead discovery was first proposed a quarter of a century ago [2], but difficulties finding and linking fragments delayed serious pursuit until 1996, when the first practical demonstration was published [3].

Goal of FBDD

The goal of FBBD is to identify one or more fragments sets, with each set comprising fragments that bind predominantly to a unique pocket or region of the desired binding site on the target protein and then to select the most optimum fragments either from each set, eventually to be linked together based on their spatial proximity to form a sufficiently potent compound or from a single such set, eventually to be grown into a sufficiently potent compounds [4]. While the goal is simple, the process whereby it can be achieved is more complex.

Many HTS hits that are sufficiently potent to be actively considered by chemistry and the vast majority of lead candidates destined for complex molecular structures comprised of multiple, interconnected ring systems on to which any number of substituent pharmacophores are grafted. These structures are complex because of their size, extent of conformational freedom and array of diverse chemical substitution [5]. A careful analysis of such structures reveals that they can be deconstructed into a set of denuded chemical building blocks, such as heterocyclic and phenyl rings, and a set of linkers comprised of amide, urea, ketone and methylene functionalities. From these basic building blocks and linkers, one can envision constructing smaller, less complex molecular structures that present only a limited number of both pharmacophores and degrees of conformational freedom.

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These structures are called fragments [4]. A fragment may be thought of as that molecular unit which occupies a single pocket or sub pocket in an enzyme such as the S1 pocket of Factor VIIa , with Sufficient potency and directionality such that the binding event can be measured and the binding mode characterized by preferably a single, defined orientation. [6].

A fragment is typically defined by the Astex Rule of 3 in which the MW is 300, and the number of hydrogen-bond acceptors, hydrogen-bond donors and rotatable bonds is each 3.25 An analysis of fragments in terms of Similog keys, which are related to 3-point pharmacophores, has suggested that a minimum of 13–20 such keys must be present in order for a binding event to be detectable by any one of the myriad techniques currently available [7].

Theoretical advantages of fragment-based drug discovery

The basic approach of fragment-based lead discovery is shown in Figure 1. After an initial fragment that binds to a protein of interest is identified, it is either elaborated or combined with other fragments to generate a hit, which is subsequently optimized to produce a lead, and a drug. Many disparate methods for finding fragments have been developed over the past few years. The strategies to transform a fragment to a lead also range considerably, from small changes to the fragment itself to dramatic substitutions and linkage with other fragments [8]. As suggested in the figure, these distinctions are sometimes more semantic than real, and in theory the same final molecule could be created by linking two fragments, optimizing a single fragment or through some combination. One of the main theoretical advantages of this approach is that there are fewer possible fragments than possible drug-sized molecules. [9]. By way of analogy, only two English words consist of one letter: 'a' and 'I'. The number of Two-letter words is larger, but still limited; however, the number of six letter words is considerable. Similarly, there are about 107 fragments with up to 12 heavy atoms (excluding 3- and 4-ring containing structures) compared with an estimated 1063 small drug-like molecules with up to 30 heavy atoms. Given that all the laboratories throughout the world are estimated to contain 'only' about 108 molecules, screening fragments would sample pharmaceutically acceptable chemical diversity space much more efficiently [10].

A second theoretical advantage of fragment-based lead discovery is that the resulting molecules are likely to have a higher 'ligand efficiency' than molecules discovered through conventional methods. An investigation of roughly 150 ligands, many of them drugs, reveals that the free energy of binding increases roughly linearly with increasing ligand size up to about 15 atoms, beyond which there is very little increase; the maximum free-energy contribution per heavy atom is roughly –1.5 kcal/mol [11].

Fragment optimization

A challenge in providing the current opinion of fragment based lead discovery is in limiting the scope of the review. For example, the c-Jun NH2 terminal kinase (JNK) inhibitors recently reported by Zhao et al [12]. began with a HTS hit (Figure 2) that qualifies as a fragment (MW = 220, 16 heavy atoms). The resulting optimization could easily be called 'fragment optimization' or 'fragment growing'. However, it is also classic medicinal chemistry, albeit with a more 'lead-like' than 'drug-like' starting point [13]. Such examples are increasingly common in the literature as fragment-like approaches permeate all aspects of medicinal chemistry and fragments become more common in HTS libraries.

The role of medicinal chemist in FBDD

The medicinal chemist plays a different role in the FBDD and HTS approach. In the FBDD approach, the medicinal chemist plays the role of a combined synthetic and structural chemical. The emphasis on informatics is greatly reduced because there is less data overall and most of it, such as from NMR or X-ray crystal structure, is visually analyzed, typically being complemented only by functional assay data on just the target itself. The emphasis on pure medicinal chemistry is also reduced, especially in the early stages of the fragment-based hit to lead (FHTL), because the overriding initial task in FHTL is to select the appropriate fragment hits to be elaborated by the appropriate synthetic methods to establish that potency, and subsequently selectivity can be increased in an efficient manner [14].

FBDD screening techniques

Nuclear magnetic resonance - Protein NMR was one of the first screening techniques applied to FBDD, known widely as "SAR by NMR". It originally required high concentrations of compound and large amounts of protein. It was also relatively slow. Recent improvements in methodology (the development of cryo-probes, miniaturization of the NMR equipment and development of multiplexed Nano-scale processes) make NMR an increasingly attractive option in FBDD [15]. The sensitivity of this approach depends on the maximum net chemical-shift perturbation that a specific ligand induces upon binding to the protein. This net perturbation is a sum of both the perturbation induced by the ligand itself upon binding and of that induced by changes in protein conformation upon ligand binding. As such, one could envision situations where the net perturbation is close to zero, even though a ligand has bound to a protein binding site with a normally sufficient level of occupancy. [16].

X-ray crystallography - The use of X-ray crystallography in FBDD has become more prevalent, fostered by the development of techniques to more rapidly find conditions for protein crystallization and robotic methods for

obtaining crystal structures. Having a ligand–protein crystal structure provides structural information about the mode of binding and gives considerable insight into the chemistry optimization process. [17].

Substrate activity screening [SAS] - This is a fragment screening strategy in which substrates for a particular target protein are identified and then optimized rapidly.[18]

For example, substrates for the cysteinyl protease cathepsin S bearing a fluorogenic group were optimized for cleavage.[19] The hydrolyzedamide bond serves as a structural anchor as to where in the protein the substratesare binding, so that enhancement of substrate activity determines what structural features improve the binding interaction off of the C-terminal side. [20].

In situ click chemistry- Several reports have appeared in which fragment-like monomers are allowed to react together in the presence of target proteins [21]. In certain cases, the rate of formation of a few possible products from a potentially large number is enhanced because of the appropriate orientation of the fragments mediated by the protein, and the resulting compounds can be potent inhibitors or modulators because they are pre-selected to be good binders [22]. This approach has been effectively applied to the [3, 2]-triazole forming reaction of azides with acetylenes, also known as click chemistry because the reactive partners appear to snap together when properly oriented. [23]

SPR spectroscopy - SPR isused to monitor the concentration of proteins at the surface of a solid support by measuring changes in refractive index. Libraries of functionalized fragments differing by virtue of the spacer length and composition as well as the linker functionality itself have been conjugated to solid support, followed by exposure to target proteins of interest. [24]. In this way, early SAR trends relative to fragment binding have been determined providing new insight for inhibitors of enzymes such asthrombin [25].

SAR by mass spectroscopy - In some cases, it is possible to observe ligand–protein complexes of fragment libraries by electro spray ionization mass Spectrometry. [26] Covalently attached fragments can be detected when they are bound onto proteins. In one variant, libraries of sulfhydryl-group (-SH) containing fragmentsare incubated with protein targets which are mutated to incorporate Cys residues near the active site [27].

Case study

PLX4032 (Vemurafenib) as a BRAF inhibitor

PLX4032 is one of the first approved drugs of which the origin can be traced back to a FBDD hit discovery. In 2002, Davies et al. reported that activating mutations (V600E) in the BRAF encoding gene were present in a significant population of malignant melanoma patients [28].

This report caused several groups to embark on a drug discovery program targeting this on cogenic mutant BRAF kinase, including a research team at the Plexxikon Inc. (a member of Daiichi Sankyo group). They opted for a modified fragment based drug discovery approach, referred to as scaffold based drug discovery. In order to identify protein kinase scaffolds, a library of 20,000 compounds (of which the molecular mass ranged between 125 to 350 Dalton) was created [29].

This library was screened at 200M on a divergent set of structurally characterized kinases. Analysis of this data resulted in the selection 238 compounds, with at least 30% inhibitory activity at 200 M for three different kinases (Pim-1, p38, and CSK). In total over 100 structures were solved containing a small molecule. In particular a 7-azaindole drew the researchers' attention since it was able to form key hydrogen bonding interactions within the active site and subsequently a set of derivatives were synthesized resulting in increased affinity. [30] The pharmacokinetic analysis in animal models of PLX4720 analogues led to the selection of PLX4032 (Vemurafenib), over PLX4720, for further clinical evaluation because of a more favorable pharmacokinetic profile.
Figure -3 [30].

Hsp90

Evotec identified novel small molecules that are potent Hsp90 inhibitors from a high throughput biochemical fragment screen. The fragment hits were rapidly optimised using two complementary strategies. Two fragments binding in distinct pockets were linked resulting in a 1,000-fold increase in potency. A third fragment was optimised using a combination of in silico analogue selection, synthesis and structure-based design. [31].

Sample of hsp90 fragment hits: Promising fragment hits were submitted to co-crystallisation and soaking experiments with the N-terminal domain of Hsp90.
Figure - 4 [31].

Fragment evolution: FROM SUB-mm TO SUB- µm in 10 compounds:

- Sub-structure searches performed against 3.8 million available compounds
- Hits docked (GOLD™), scored and visually inspected for key interactions
- Compounds purchased and tested
- Analogues synthesised and tested
- Virtual library designed and docked. Design focussed on introducing interaction with helical pocket
- Synthesised compounds show further increases in potency

3. BACE case study

Fragment approach delivers novel starting points for challenging target. BACE1 is a protein implicated in the pathogenesis of Alzheimer's disease. Evotec has identified novel fragment inhibitors of BACE1 from a biochemical fragment based screen. Co-crystal structures for fragments and optimised inhibitors have been obtained and form the basis for subsequent structure-guided medicinal chemistry program [31].

Evotec's approach to identifying novel bace1 inhibitors includes:

- A high throughput fragment screen of Evotec's diverse fragment library, utilising Evotec's sensitive FCS plus functional BACE1 assay
- Counter-screening with a secondary assay for hit validation
- Confirmation of ligand binding by SPR experiments using an Inhibition in Solution Assay (ISA)
- In vivo model for rapid testing of lowering drugs for driving medicinal chemistry program.

Figure 1. (The basic concept of fragment-based lead discovery. The blue ovoid and red rectangle represent fragments that bind to the target protein. These can be linked or expanded to produce high-affinity ligands.)

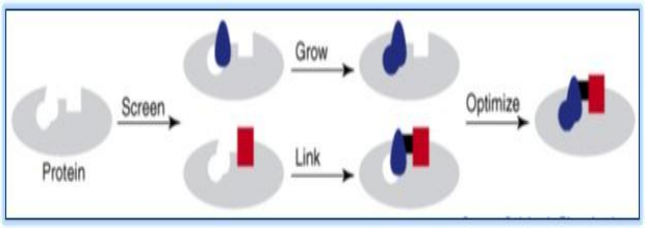


Figure 2. Structure

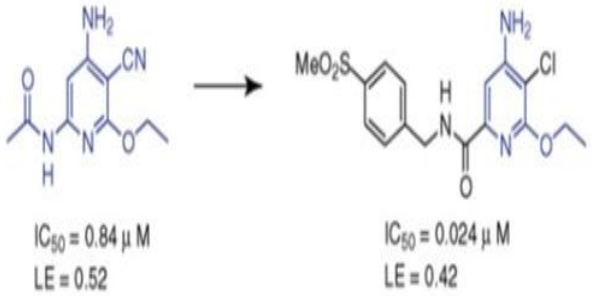
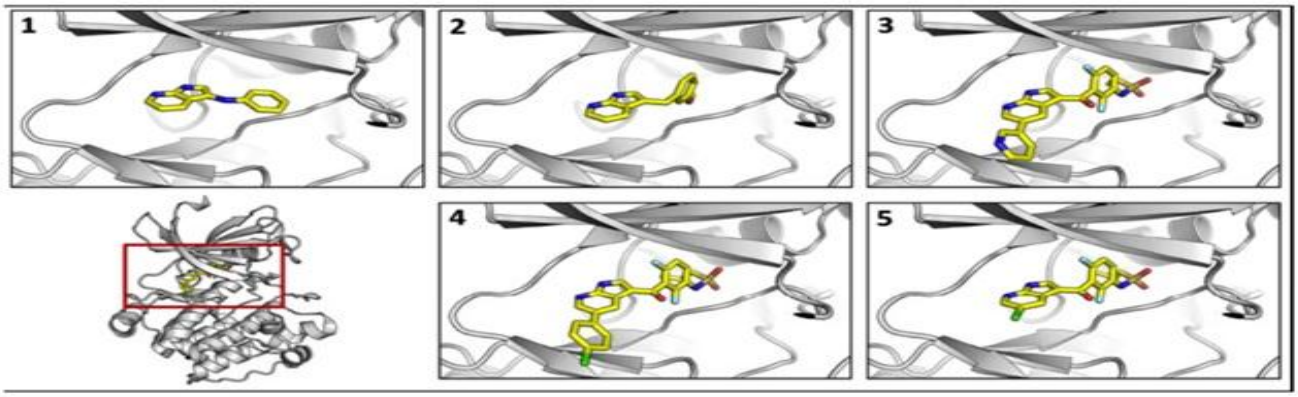


Figure 3. (High concentration screening of scaffold molecules against a kinase revealed a series of hit molecules.)



A 7-azaindole scaffold, was able to bind to the active site of the kinase domain [frame 1]. Scaffold molecule would be modified and gained in potency [frame 2]. In several rounds of optimization the scaffold was grown into a more potent molecule [frame 3]. In final round several molecules with high affinity were identified [frame 4]. PLX4032 was selected for clinical development over a more potent derivative, PLX4720 [frame 5] due to its superior Pharmacokinetic properties.

Figure 4.

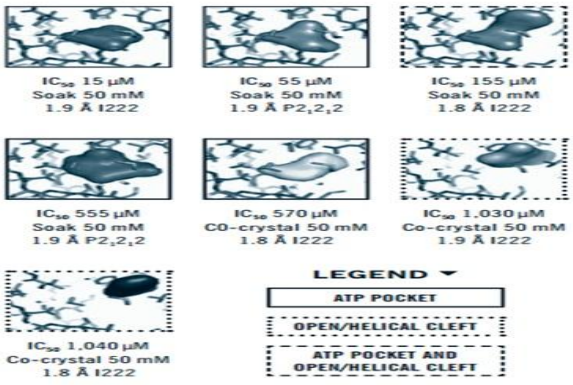


Figure 5.

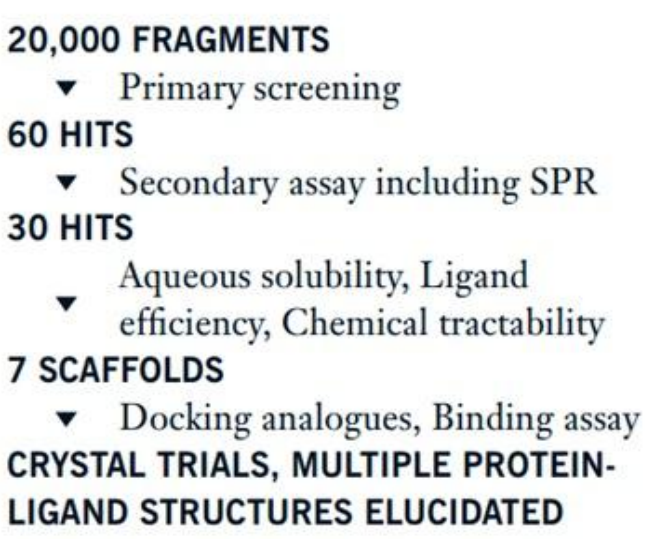


Figure 6.

FRAGMENT	1	1.1	1.2
BACE1 IC_{50}	800 µM	16 µM	7 µM
Aβ secretion IC_{50}	---	---	12 µM
LE	0.29	0.28	0.29
MW	226	349	363
clogP	1.52	3.75	4.63
TPSA	64	64	73
Lipinski violations	0	0	0
Co-crystal	1.8 Å	2.1 Å	2.4 Å

Structure-based design using the elucidated structure of the Fragment1:BACE1 complex. One cycle of optimisation improved the potency of a fragment in series 1 by a 100-fold, from an 800 µM fragment to a 7 µM inhibitor. This compound is functionally active in a cellular Aβ cleavage assay at 12 µM IC_{50}. The co-crystal structure of the compound was solved at 2.4 Å resolution, offering an excellent starting point for further optimisation.

CONCLUSION

Fragment based drug design (FBDD) is a powerful and widely used drug discovery approach. It involves the identification of low molecular weight chemical fragments and their optimization into lead compounds. The success in fragment based drug discovery to recent technological advancements in fragment screening technologies. X-ray crystallography and NMR based screening strategies were particularly successful in identifying highly ligand efficient fragment hits that could serve as scaffolds for the generation of potent lead compounds for many targets. The recent use of technologies like SPR based biosensor assay made possible the high-throughput label-free screening of fragments against membrane proteins. Advantages of experimental fragment screening methodologies; its applicability is limited because of the cost associated with experiments, high protein and fragment requirement, low throughput nature and limited target applicability. Fragment-based lead discovery needs better strategies for linking and expanding fragments without generating unacceptably large molecules. The impact of FBDD has been greatly accelerated by the deployment of rapid iterative structure-determination the synergies between FBDD, protein structure determination and rapid chemical synthesis accounts for FBDD's efficiency and productivity, positioning fragment-based approaches. FBDD will play a larger role in drug discovery by improving the quality of lead compounds, the efficiency in lead optimization and the productivity in finding better drugs that benefit patients.

Abbreviations

FBDD = Fragment Based Drug Design
HTS = High Throughput Screening
NMR = Nuclear Magnetic Resonance
SAS= Substrate activity screening
SAR= Structure-Activity. Relation
FHTL= fragment-based hit to lead
MW= Molecular weight

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