

## Will Smaller be Better? —Accelerated Lead Generation

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### Abstract

High-throughput screening (HTS) based lead generation is the default approach to the majority of pharmaceutical organization. The fact that HTS can often produce “low wattage” resulted in pursuing new strategies to optimize efficiency of the process. Recently, alternative lead generation strategies evolve and expand in responding to such needs. Differing from conventional HTS-based approach, new strategies use smaller small molecules (SSM) to serve as the entry point of lead generation. Application of SSM-based approach in various targets has demonstrated that it can generate leads in cost-effective and timely manner, and therefore has received increasing attention in drug discovery community.

### Introduction

Lead generation is an established platform that is used to identify, validate, and refine hits in the early phase of drug discovery. Having multiple milestone criteria from potency, selectivity to ADME and toxicity, lead generation sets the basis for lead optimization and clinical candidate development. The need to provide drug-like leads have shown increasing urgency since drug discovery process evolves from serendipitous approach to rational drug design during the last two decades. Introduction of new technologies such as combinatorial chemistry and high-throughput screening (HTS) has largely enhanced capacity to satisfy such needs by providing hundreds of thousands of compounds and performing screening routinely against a variety of targets. It is estimated that number of drug candidates from HTS campaign increased from around 20 in 1995 to nearly 70 in 2000.[1] On the other hand, it is increasingly recognized that a fair percentage of potent but unsuitable hits discovered in HTS proven difficult or impossible to be advanced for further exploration. The cost for massive growth in compound collection and screening is barely matched by the corresponding increase in the number of either new chemical entities launched or drugs marketed. To curb costs and improve success rate, calls for new strategies other than HTS-based lead generation approach have been addressed into a new level of awareness and importance.

Alternative lead generation strategies have received increasing attention in drug discovery community since early 1990s. Success of “SAR by NMR” method for fragment-based lead discovery in 1996 was a significant step, [2] which triggered widespread interest and has been followed by numerous examples since then. Recently, a group of scientists at Plexxikon reported discovery of a family of phosphodiesterase (PDEs) inhibitors through scaffold-based drug design.<sup>3</sup> In both cases, only small libraries of compounds are required to generate quality leads. Since both leads were refined from hits (< 250 ~ 350 Da) smaller than conventional HTS-based hits, they may be termed smaller small molecule (SSM) based lead generation. SSM-based lead

generation, as well as another noteworthy approach via virtual screening (VS), [3] exhibit a common feature of taking advantage of the advances in new technologies and informatics. Their continued success will result in far-reaching consequences to mindset in lead generation.

### SSM-Based Lead Generation

Contrast to HTS-based lead generation where hits are usually identified from libraries with over 100K of compounds, SSM-based lead generation requires much less compounds, typically less than 10K. It is the SSM hit, most frequently referred as "fragment" in literature, that allows this new strategy to generate leads in such cost-efficient and timely manner. SSM-based hits are weakly active ligands (10  $\mu\text{M}$ -mM). Their activities can only be detected at high concentration (20-400  $\mu\text{M}$ ) by highly sensitive instrumentation such as nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography. Advantage of SSM-based over conventional HTS-based approach may be present in following aspects.

#### 1.Ligand Efficiency

The binding energy of a ligand per non-hydrogen atom, or ligand efficiency (LE) is a useful parameter in lead assessment.[4, 5] Maintenance or improvement of relatively high LE in the process of lead generation and optimization has gained increased awareness recently due to its impact on the developability of clinical candidates. [6] Despite low affinity SSM-based leads exhibits, their LE is usually higher than that of HTS leads. For an instance, LE of SSM-based lead with molecular weight (MW) of 160 Da and dissociation constant (Kd) of 800  $\mu\text{M}$  is calculated to be 0.22, comparing with 0.28 for an HTS lead with MW of 400 Da and Kd of 1  $\mu\text{M}$ . [7] Therefore, SSM-based leads may have intrinsic advantage in terms of lead quality. By contrast, HTS-based leads are more likely to confound further optimization, because lower LE could attribute to part of unfavorable physicochemical properties and pharmacokinetic profile,

#### 2.Chemical Space

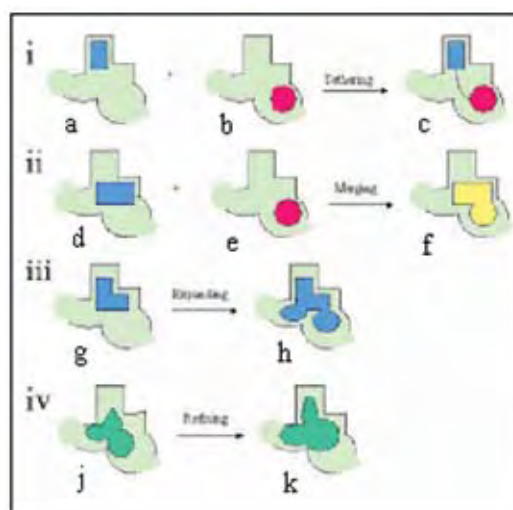
Chemical space may be viewed as a sphere where elements are at the center, and the number of possible molecules within a layer of certain thickness exponentially increases as radius (MW) extends towards surface. Although library synthesis and HTS capabilities have been significantly enhanced since its inception, it is still impossible for pharmaceutical companies to match the size of chemical universe with their compounds collection. One practical solution is to build up diversified subsets besides increasing the sizes of collections, which has been a trend becoming visible. At the same time, the question of whether it can be tackled by

examining the sphere of chemical universe a bit deeper has raised widespread interests.

It is estimated that there are only approximately 14 million compounds if molecular weight cut-off of compounds is set to 160 Da, [8] while the number of compounds in chemical universe could reach an appalling number of 1060. [9] Pursuing the SSM-based approach is then a reasonable and practical strategy. It might be fruitless to screen SSMs twenty years ago. However, with the introduction of highly sensitive instrumentation and robust crystallography, SSM-based approach has emerged and progressed many quality leads into clinical trials.

#### 3.Molecular Complexity

One of obvious differences between SSM-based and HTS-based lead generation is their molecular complexity. Molecular complexity can be measured based on the calculated structural complexity scores, and its effect on success rate was studied by comparison marketed drugs and their leads. [10] While certain level of molecular complexity is necessary for detectable biological activity, [11] increased complexity of lead molecules often means difficult disentanglement in structural-activity relationship (SAR) study.

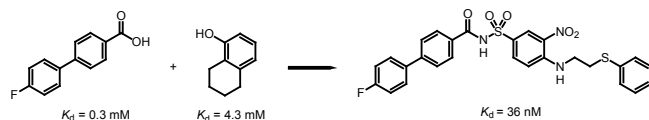


**Figure 1. SSM-based (i, ii, and iii) vs. HTS-based (iv) lead generation. (i) Fragment-based approach via tethering of two fragments; (ii) Fragment-based approach via merging of two fragments; (iii) Scaffold-based approach via expanding hits into leads; (iv) HTS-based approach.**

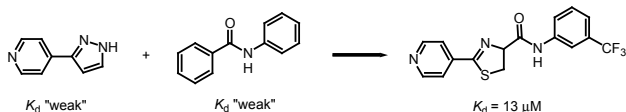
Leads with less complexity attribute to high success rates in lead generation via both higher hit rates and more available chemical space. Schuffenhauer and co-workers recently reported their observation of substantially higher hit rates from

fragment-based than HTS-based screening (10–1,000 fold). [12] It is also known that success rate in hit-to-lead favors alternative approaches (60% vs. 40% for HTS approach). [13] Simpler leads would provide more diversified chemical space in lead generation, easier manipulation in lead optimization, and potentially better pharmacokinetic properties such as oral bioavailability. [14]

SSM-based approaches choose smaller small molecules with high LE as the entry point for lead generation, which provides more chemical space and less molecular complexity towards lead optimization (LO) and clinical candidate selection (CCS). This concept, along with HTS-based approach, is illustrated in figure 1. Approaches i and ii are two of widely applied fragment-based lead generation strategies, where low-affinity fragments are either linked or merged to provide hits or leads for further exploration. Clinical candidate ABT-737 was developed from leads through application of the “SAR by NMR” method [15, 16] (Scheme 1). Novel Jun kinase-3 (JNK3) inhibitors were obtained by merging two weak fragments into an active hit [17] (Scheme 2). In both cases, fragment-based approach yielded quality hits or leads while conventional HTS-based approach failed.



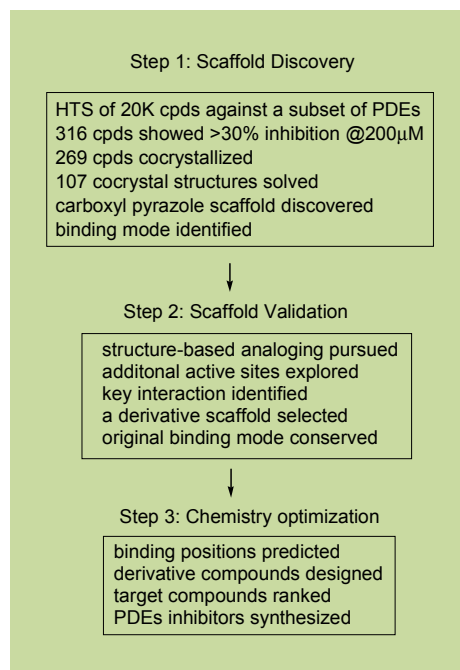
**Scheme 1. Fragment Tethering at Abbott, BCL-XL**



**Scheme 2. Fragment Merging at Vertex, JNK3**

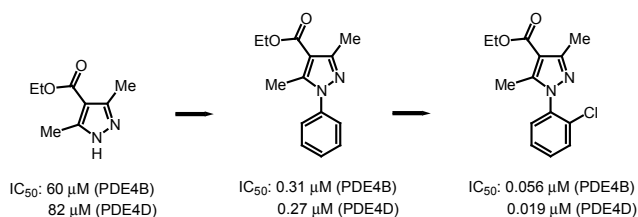
While fragment-based lead generation are well covered in recent reviews, [18, 19, 20] there are only few seen today that reveal the importance of scaffold to this emerging strategy in lead generation process. One of the best examples came from Plexxikon where a family of phosphodiesterase (PDEs) inhibitors was discovered by cocrystallography and scaffold-based drug design.[3] Scaffold-based lead generation approach consists of three steps, that is, scaffold discovery, scaffold validation, and chemistry optimization (Figure 2).

The strategy used at Plexxikon can be classified into the third approach illustrated in Figure 1. A low-affinity hit was first identified; it was expanded with its binding mode conserved,



**Figure 2. Scaffold-based drug design: from low-affinity hits to potent leads in 3 steps.**

and it was then modified to yield potent PDE inhibitors (Scheme 3). It is noteworthy that compounds screened in scaffold-based approach (M.W. < 350 Da) are slightly larger than that in fragment-based approach (M.W. < 250 Da). Although the “expanding” strategy is also common in fragment-based approach, the latter may encounter more challenges such as possibly multiple binding modes because small fragments are more likely to be flipped over in binding pockets, which reduces predictability of SAR study and efficiency of optimization. In addition, scaffold-based approach developed at Plexxikon is a combination of high-concentration screening (HCS) and



**Scheme 3. Scaffold-based lead generation at Plexxikon, PDEs**

X-ray cocrystallography, which distinguishes it from fragment-based approach where NMR or X-ray fragment screening is performed in the first round. It seems unfruitful to define whether molecular weight of fragments should be within

range of 120-250 or 120–350 Da. In my opinion, it would be appropriate to include both approaches as SSM-based lead generation for direct comparison with HTS-based lead generation.

## Summary

The role of SSM-based lead generation approach is becoming increasingly acknowledged in drug discovery community. As an alternative to HTS-based approach, it often yields quality leads rapidly because of intrinsic advantages existed in SSM-based hits. Continued success of SSM-based approach would lead to an integrated multi-dimensional process, where many crucial drug features such as physicochemical properties and absorption, distribution, metabolism, excretion, toxicity (ADMET) are considered early to reduce attrition in the costly clinical phases.

SSM-based lead generation strategy inevitably has its limitations. NMR screening methods consume a lot of protein; X-ray crystallographic screening needs crystals, which limits the number of applicable targets. Solubility of SSMs is also of concern because screens are performed at high concentration. In addition, more chemistry efforts may be needed to bring low-affinity into the range of HTS-based hits. Nevertheless, many of these shortcomings could be overcome by development of new methods, new technologies, and the advance of computation. Considering the remarkable progress it made during the past decade, SSM-based approach will undoubtedly continue to evolve and expand to be a critical lead generation strategy in modern drug discovery.

## References

1. France, D. Beyond biomolecular screening: A multi-paradigm approach to oncology hit-finding. AACR annual meeting, Washington D.C. 2006
2. Shuker, S. B.; Hajduk, P. J.; Meadows, R. P.; Fesik, S. W. Discovering high-affinity ligands for protein: SAR by NMR. *Science* 1996, 274, 1531-1534.
3. Laird, R. E.; Blake, J. F. *Curr. Opin. in Drug Disco. Dev* 2004, 7(3), 354-359.
4. Hopkins, A. L. et al. Ligand efficiency: a useful metric for lead selection. *Drug Discovery Today* 2004, 9, 430-431.
5. Kuntz, I. et al. The maximal affinity of ligands *Proc. Natl. Acad. Sci. U. S. A.* 1999, 96, 9997-10002.
6. Abad-Zapatero, C. and Metz, J. T. Ligand efficiency indices as guideposts for drug discovery. *Drug Discovery Today* 2005, 10, 464-469.
7. Lyne, P. Rational and structural based approaches for lead generation. AACR annual meeting, Washington D.C. 2006
8. Fink, T., et al. Virtual exploration of the small-molecule chemical universe below 160 Daltons. *Angew. Chem. Int. Ed. Engl.* 2005, 44, 1504-1508.
9. Martin, Y. C. Challenges and prospects for computational aids to molecular diversity. *Perspect. Drug. Disco. Design.* 1997, 7-8, 159-172.
10. Hann, M. et al. Molecular complexity and its impact on the probability of finding leads for drug discovery. *J. Chem. Inf. Comput. Sci.* 2001, 41, 856-864.
11. Schuffenhauer, A. et al. Relationship between molecular complexity, biological activity, and structural diversity. *J. Chem. Inf. Comput. Sci.* 2006, 46, 525-535.
12. Schuffenhauer, A. et al. Library design for fragment based screening. *Curr. Top. Med. Chem.* 2005, 5, 751-762.
13. Pass, M. Chemical approaches to lead generation. AACR annual meeting, Washington D.C. 2006
14. Vieth, M. et al. Characteristic physical properties and structural fragments of marketed oral drugs. *J. Med. Chem.* 2004, 47, 224-232.
15. Olterdorf, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumors. *Nature*, 2005, 435, 677-681.
16. Petros, A. M. et al. Discovery of a potent inhibitor of the antiapoptotic protein Bcl-xL from NMR and parallel synthesis. *J. Med. Chem.* 2006, 49, 656-663.
17. Fejzo, J. et al. Application of NMR screening in drug discovery. *Curr. Top. Med. Chem.* 2003, 3, 81-97
18. Hajduk, P. J. and Greer J. A decade of fragment-based drug design: strategic advances and lessons learned. *Nat. Rev. Drug Discovery* 2007, advanced online publication.
19. Carr, R. A. E. et al. Fragment-based lead discovery: leads by design. *Drug Discovery Today*. 2005, 10(14), 987-992.
20. Erlanson D. A. Fragment-based drug discovery. *J. Med. Chem.* 2004, 47(14), 3463-3482.