



**Trip Report for**  
**“Drug Discovery Chemistry”**  
**La Jolla, California**  
**May 13-16, 2007**

**John W. Lippert III, Ph.D.**

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**Abstract:** *Cambridge Healthtech Institute’s “Drug Discovery Chemistry” was held at the Hilton Torrey Pines in La Jolla, California, on May 13-16, 2007. About 200 attendees from biotech and pharmaceutical companies as well as academic institutions attended this conference. This symposium featured four different tracks: Track 1: Fragment-Based Drug Discovery; Track 2: High-Throughput Organic Synthesis and Purification; Track 3: G-Protein Coupled Receptor Drug Discovery; and Track 4: Protein-Protein Interactions as Drug Targets. Included below are some of the highlights from Track 1: Fragment-Based Drug Discovery, and Track 4: Protein-Protein Interactions as Drug Targets.*

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## **“Rapid Compound Production: Microwave-Assisted Synthesis, Work-up, and Purification”**

*Farah Mavandadi, Ph.D., Product Manager Marketing, Microwave Systems, Biotage*

This presentation provided an overview covering the scope and advantages of the “discovery chemistry tool kit” offered at Biotage for organic chemists. Their toolbox includes the following items:

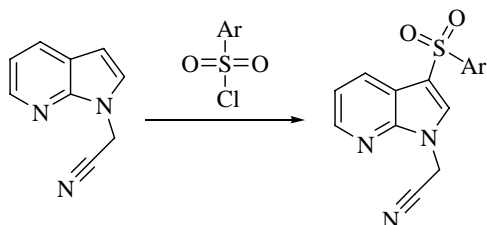
- Microwave Synthesis
  1. Extremely fast reactions
  2. High yield and purity
  3. Enables “impossible” reactions
  4. Reproducible
- Solid-Bound Reagent and Scavenger SPE
  1. Simplifies purification
  2. Facilitates reaction chemistry
- Automated Flash Purification
  1. 1-10 cartridges in unattended operation
  2. TLC to gradient method development
- Rapid Solvent Evaporation
  1. Organic solvents and water
  2. Flexible formats
  3. Flow and batch mode
  4. Effective re-dissolution

Through the use of the items mentioned in the above “toolbox”, Biotage offers a synergy of accelerated synthesis, accelerated work-up, and accelerated purification. Two of the four items listed above, microwave synthesis and solid-bound reagents and scavengers are discussed below.

Microwave Assisted Organic Synthesis (MAOS) has several advantages over conventional reactions in that the microwave allows for an increase in reaction rate, rapid reaction optimization, and rapid analogue synthesis. It also uses both less energy and solvent, and it enables difficult compound synthesis.

The microwave assisted sulfonylation of 7-azaindoly-1-yl acetonitriles provides an example of how microwave assisted chemistry has advantages over conventional chemistry (Scheme 1).

### Scheme 1



Conventional: 125 °C / 18-20 h; 11-18%  
 $\text{tmw} = \text{tconv} \times 0.5[(\text{tmw} - \text{tconv}) / 10 \text{ }^\circ\text{C}]$   
 $\text{tmw} = 1440 \text{ min} \times 0.5[(200 \text{ }^\circ\text{C} - 120 \text{ }^\circ\text{C}) / 10 \text{ }^\circ\text{C}]$   
 $\text{tmw} = 5.6 \text{ min}$   
Microwave: 200 °C / 5 min; 30-40%

This reaction also serves as a good example for the rapid optimization of reaction conditions, which was all done in the microwave. At 5 min a reaction, 45 sulfonylations were carried out in ~ 4 h. Optimal reaction conditions showed that 1 eq. of AgOTf was needed along with the  $\text{ArSO}_2\text{Cl}$ , and that nitrotoluene was the preferred solvent of choice.

In general, drug discovery can be broken down into five steps:

1. Target and Synthesis Design
2. Reaction
3. Work-up – usually extraction and evaporation
4. Purification – usually chromatography
5. Spectral Analysis Registration

The main bottlenecks in synthetic organic chemistry are at the work-up and purification stages. By using Solid Supported Organic Synthesis (SSOS), chemists now can minimize the time spent on reaction work-up (filtration) using resin bound reagents or scavengers (Table 1 and 2, respectively).

**Table 1**

<b>BIOTAGE RESIN REAGENTS</b>		
Bound Reagent	Solution Analog	Application
PS-TsCl	p-toluenesulfonyl chloride	Catch and Release
MP-TsOH	p-toluenesulfonic acid	Catch and Release
PS-DIEA	Hindered Tertiary Amine	Amine Base
PS-NMM	N-methyl morpholine	Non-benzylic base
PS-DMAP	DMAP	Catalyst, Catch and Release
MP-Carbonate	Ammonium Carbonate	Base, Catch and Release
PS-Triphenylphospine	Triphenylphosphine	Mitsunobu/Wittig/Halogenation
PS-PPh <sub>3</sub> -Pd	Triphenylphosphine-Pd (0)	Palladium Catalyst
PS-Carbodiimide	DCC	Coupling Reagent
PS-HOBt (HL)	HOBt	Coupling Reagent
MP-Borohydride	Sodium Borohydride	Reducing Reagent
MP-Cyanoborohydride	Sodium Cyanoborohydride	Reducing Reagent
MP-Triacetoxymorohydride	Sodium Triacetoxymorohydride	Reducing Reagent
MP-TsO-Tempo	TEMPO	Oxidizing Reagent

**Table 2**

<b>BIOTAGE POLYMERIC SCAVENGERS</b>		
Electrophile	PS-Trisamine MP-Trisamine	Acyl halides, Sulfonyl Halides, Isocyanates
	MP-Carbonate	Carboxylic Acids, Phenols
	PS-Tosylhydrazide	Aldehydes, Ketones
	PS-Thiophenol	Alkylating Agents
	PS-Triphenylphosphine	Alkyl Halides
Nucleophile	PS-Isocyanate MP-Isocyanate	1°, 2° amines, hydrazine
	PS-Benzaldehyde	1° amines
	PS-Tosyl Chloride	Anilines, Alcohols
	MP-Tosic Acid	Amines, Anilines
Metal	MP-TMT	Pd (0)
	MP-DEAM	Ti (IV), Sn (IV), Boronic Acids

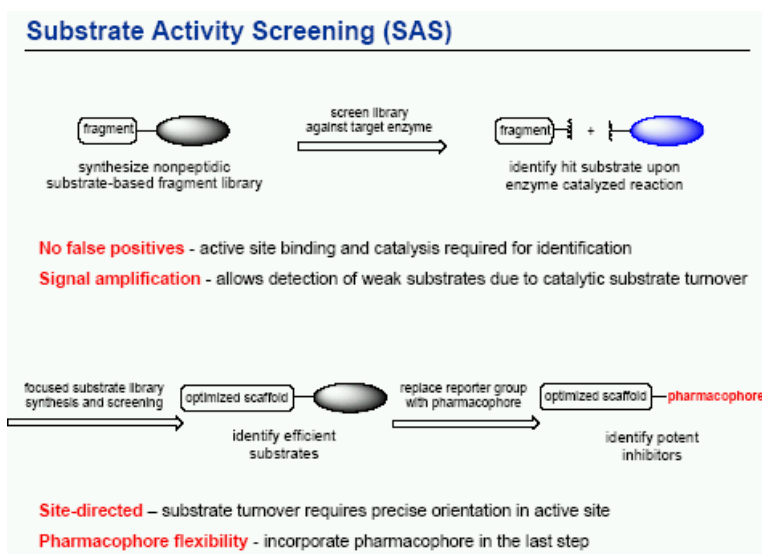
**“Substrate Activity Screening (SAS): A Fragment-Based Method for the Identification of Nonpeptidic Protease Inhibitors”**

**Andrew W. Patterson, Researcher, Chemistry, University of California, Berkeley**

This presentation focused on the solid-phase synthesis of nonpeptidic substrate-based fragment libraries for the purpose of identifying protease inhibitors. This work has been carried out within the Ellman Group at the University of California in Berkeley.

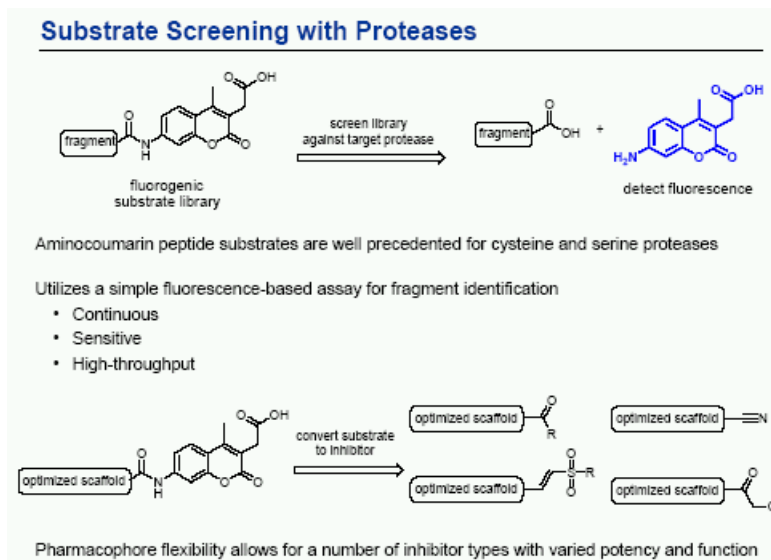
A new fragment-based method for the rapid development of novel and distinct classes of nonpeptidic protease inhibitors, Substrate Activity Screening (SAS) was presented (Figure 1). This method consists of three steps: (1) a library of *N*-acyl aminocoumarins with diverse, low molecular weight *N*-acyl groups was screened to identify protease substrates using a simple fluorescence-based assay, (2) the identified *N*-acyl aminocoumarin substrates were optimized by rapid analogue synthesis and evaluation, and (3) the optimized substrates were converted to inhibitors by direct replacement of the aminocoumarin with known mechanism-based pharmacophores.<sup>4</sup>

**Figure 1**



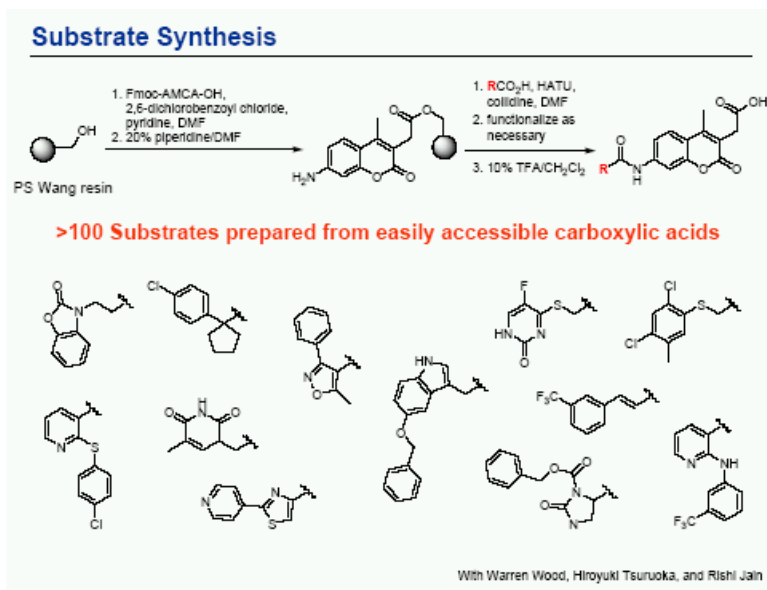
As mentioned above in step 1, the initial screening of the synthetic chemical library against the target protease uses the aminocoumarin (AMCA) group, which allows for monitoring by fluorescence (Figure 2). Upon identification of an optimized scaffold from an initial fragment, the carboxylic acid moiety is converted into an inhibitor with potency and functionality via a mechanism-based pharmacophore.

Figure 2



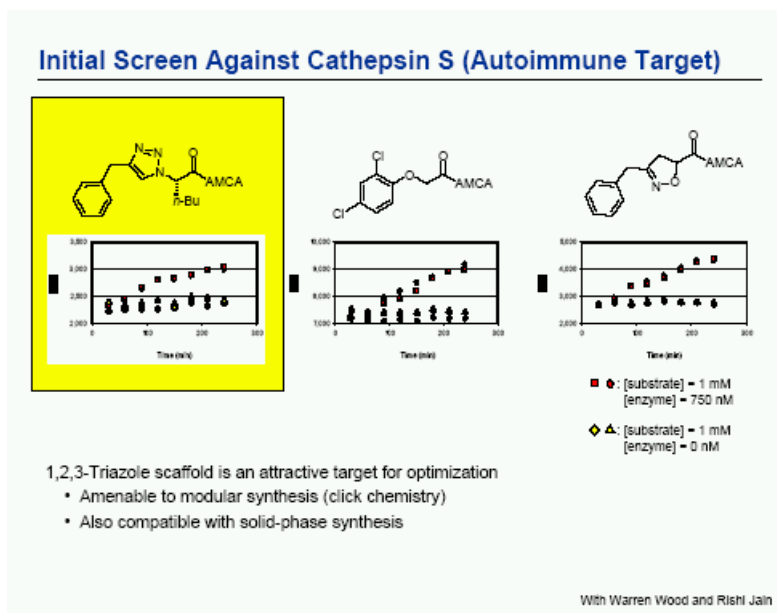
An example of a substrate synthesis to produce a fragment library using this methodology is shown in Figure 3. The library listed below produced a large number of substrates for screening purposes.

Figure 3



Initial screening results against Cathepsin S (autoimmune target), of the above library, identified the 1,2,3-triazole scaffold as an attractive target for further optimization (Figure 4).

Figure 4



A library of triazoles was then synthesized, screened, and modified by converting the eastern portion of the molecule into an aldehyde group.

Replacement of the aminocoumarin group with a hydrogen atom, which is the minimal mechanism-based pharmacophore for cysteine proteases, provides an aldehyde group.<sup>4</sup> Upon binding of the inhibitor in the active site and addition of the active-site cysteine thiol to the aldehyde, a tetrahedral adduct is formed, which is the analogue of the transition-state.<sup>4</sup> Based on the transition-state theory (Equation 1), good correlation between  $\log(K_m/k_{cat})$  and  $\log(K_i)$  is expected for compounds in related chemical series.<sup>4</sup> (both  $d$  and  $k_{un}$  should remain constant,  $d$  provides a measure of the effectiveness of the mechanism-based inhibitor as a transition state analogue and  $k_{un}$  equals the rate of hydrolysis for the uncatalyzed reaction.)<sup>4</sup>

### Equation 1

$$\log(K_i) = \log(K_m/k_{cat}) + \log(dk_{un})$$

The substrates with the greatest cleavage efficiency for each series should therefore provide the highest affinity inhibitors.<sup>4</sup>

For further information on this and other related topics see the references below:

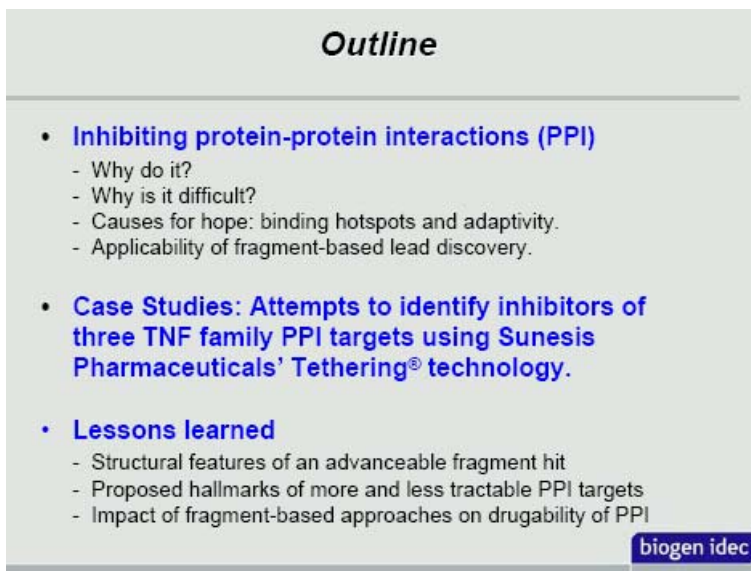
1. Wong, *et al.* *Tetrahedron Lett.* **1996**, 37, 6029.
2. Sharpless, K. B. *et al.* *Angew. Chem. Int. Ed.* **2002**, 41, 2596.
3. Meldal, M. *et al.* *J. Org. Chem.* **2002**, 67, 3057.
4. Wood, W. J. L. *et al.* *J. Am. Chem. Soc.* **2005**, 127, 15521.
5. Abbenante, G. *et al.* *Med. Chem.* **2005**, 1, 71.
6. Patterson, A. W. *et al.* *J. Med. Chem.* **2006**, 49, 6298.

## “Fragment-Based Screening Against Protein-Protein Interaction Targets”

Adrian Whitty, Ph.D., Director, Physical Biochemistry, Drug Discovery, Biogen Idec, Inc.

This presentation discussed some of the work being done at Biogen Idec, regarding fragment-based screening against protein-protein interactions. Figure 1 illustrates the general outline of his presentation.

Figure 1



The conversion of a protein-protein interaction (PPI) into a protein-small molecule interaction is financially attractive to pharmaceutical and biotech companies, due to the financial rewards. These small molecule inhibitors of PPIs are difficult to obtain because PPI interfaces are relatively large and flat. Furthermore, they typically lack a deep cleft or pocket that allows multiple interactions to converge upon a ligand with a small molecular volume.

In spite of these difficulties, there is a cause for hope in this realm of drug discovery due to the fact that PPI binding energy is concentrated in binding ‘hotspots’ (particular residues of the protein). Additionally, PPI surface adaptability increases the potential for small molecule binding. Furthermore, protein binding sites are not readily predicted due to the conformational rearrangement that the target protein undergoes in the bound conformation.

The overall implications of binding hotspots and structural adaptivity of PPI are listed below:

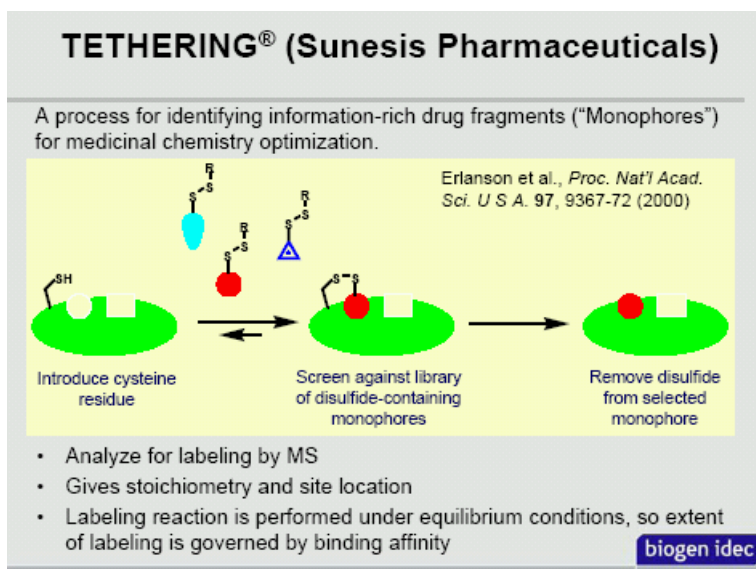
- By binding to an energetic hotspot, a small molecule might access substantial binding energy
- A small molecule with the right structure can potentially induce the formation of a sizable cavity, thus creating its own binding site. The energetic cost of the rearrangement must be small relative to the increased interaction energy generated between the protein and the small molecule.

- Researchers cannot yet predict such interactions based on the structure of the target alone. An experimental screen is required. (a fragment-based approach to lead ID is particularly suitable).

The idea behind the application of fragment-based lead identification is that one must identify small molecular fragments that bind weakly to pockets on the protein surface in an effort to link or grow them to combine multiple favorable interactions within a single molecule that binds more strongly.

A case study directed at TNF- $\alpha$  was then discussed, which used Sunesis's Tethering<sup>®</sup> technology (Figure 2). This involves use of a disulfide linkage to attach the fragment of choice. TNF- $\alpha$  was chosen for this study for several reasons including the fact that it was a highly validated target, industry high throughput screening had failed, and no attractive anti-TNF-small molecules have been reported.

**Figure 2**



Based on this tethering approach, a summary of the conclusions from the work on small molecule inhibitors of TNF family PPI are illustrated in Figure 3.

Figure 3

**Summary of Conclusions from Work on SM Inhibitors of TNF family PPI**

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**Druggability is largely a property of a given target and a set of compounds, rather than a deficiency in Lead ID technologies**

- Existence of pre-formed or inducible cavity of appropriate dimensions is essential (for conventional-drug-like molecules)
- For potency and selectivity, recognition must include both polar and hydrophobic contacts
- 'Advanceability' of hits can be evaluated on an objective basis
- Adaptivity of protein is key to PPI druggability
- Druggability should be predictable from structure

**Fragment-based Lead ID can improve odds for borderline druggable targets**

- Postulate that outcome of fragment-based screen provides good measure of druggability

**Identifying and eliminating false positive hits is key**

- We and others (e.g. Shoichet, UCSF) have established a range of biochemical and biophysical approaches to do this

(Whitty & Kumaravel, *Nat. Chem. Biol.* 2, 112-118 (2006))

**biogen idec**

In summary, the directions for future research were addressed:

- There is much we still do not understand about protein-small molecule recognition and the factors required for strong, specific binding; (1) structural or computational approaches to predicting hotspots and adaptive sites on protein targets would be extremely valuable, (2) elucidating the topological rules for a “druggable” binding site would help identify more promising targets on structural grounds
- Establishing the mechanism of action for weak inhibitors is difficult; artifacts abound and better methods are needed
- Increasing the size and complexity of drug candidates beyond conventional ‘druglike’ chemical space might substantially improve our ability to develop drugs against PPI targets

For further information on this and other related topics see the references below:

- Arkin, M.R. *et al.*, *Proc. Nat's Acad. Sci. USA* **2003**, *100*, 1603-1608.
- Rees, D.C. *et al.*, *Nat. Rev. Drug Disc.* **2004**, *3*, 660-672.
- Whitty & Kumaravel, *Nat. Chem. Biol.* **2006**, *2*, 112-118.

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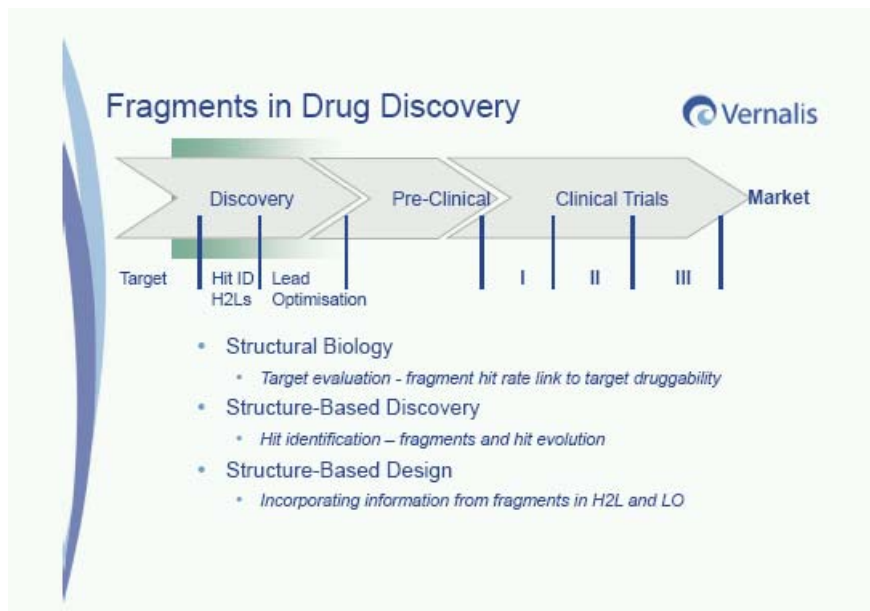
### “Hsp90 Inhibitors – Integrating Fragments into Structure Based Medicinal Chemistry”

*Martin J. Drysdale, Ph.D., Head, Chemistry, Vernalis R&D*

This presentation focused on the synthesis of Hsp90 inhibitors through the use of fragments into structure based medicinal chemistry. An outline of the topics covered is illustrated in Figure 1.

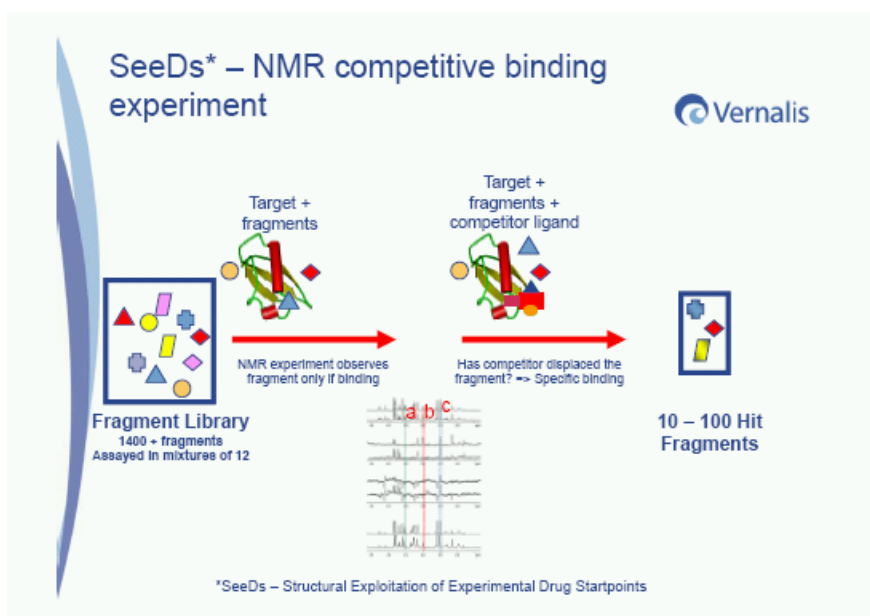


**Figure 3**



The overall concept of screening fragment libraries incorporates several different experimental approaches which each have strengths and limitations. These include X-ray crystallography, NMR, Biacore, and enzyme assays. Vernalis employs a unique NMR experiment known as SeeDs (Structural Exploitation of Experimental Drug Startpoints). This process involves a competitive binding experiment with the desired fragment library (Figure 4).

**Figure 4**



A majority of the parameters surrounding the fragment library design at Vernalis are listed in Figure 5.

**Figure 5**

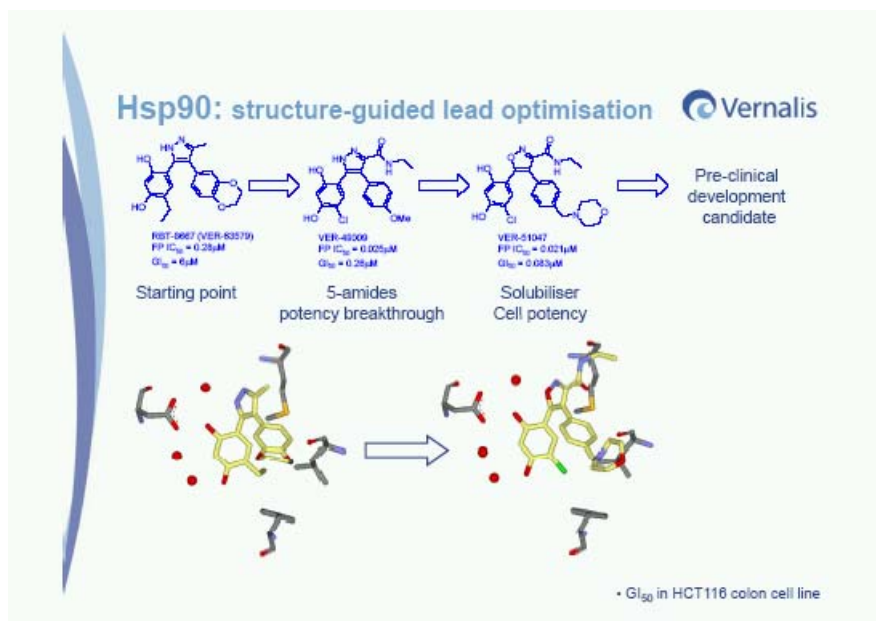


The Hsp90 program at Vernalis was pursued because it was a highly validated target. Some of the reasons are illustrated below:

- Molecular chaperone involved in protein folding
- Overexpressed in human tumors
- Essential for stability and function of many oncogenic ‘client’ proteins eg erbB2, Raf-1, CDK4, polo-1, met, mutant p53, HIF1 , estrogen/androgen receptors, telomerase hTERT
- Inhibition likely to block all ‘six hallmark traits’ of cancer; evading apoptosis, self-sufficiency in growth signals, sustained angiogenesis, unrestricted proliferation and tissue invasion & metastasis
- Proof of concept for therapeutic selectivity demonstrated in human tumour xenograft models
- First-in-class inhibitor 17-AAG now showing evidence of biological and clinical activity at well-tolerated doses

After identifying the fragments that bind to Hsp90 and designing a fragment library both X-ray crystallography and NMR spectroscopy (SeeDs) along with medicinal chemistry allowed the structure-guided lead optimization to afford a pre-clinical development candidate (Figure 6).

Figure 6



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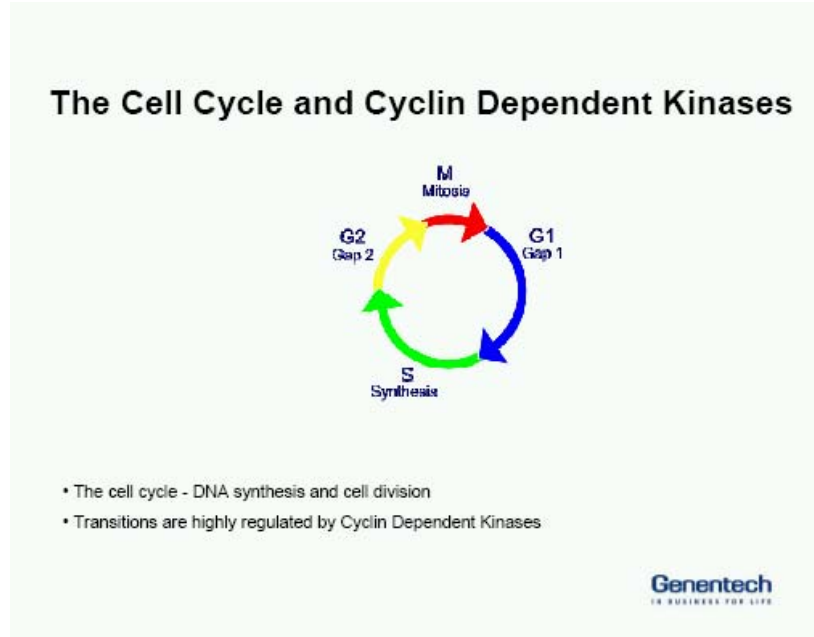
**“CDK2/CYCLIN Inhibitors: Targeting the CYCLINA Recruitment Site with Small Molecules Derived From Peptides Leads”**

*Daniel Sutherlin, Ph.D., Scientist, Medicinal Chemistry, Genentech Inc.*

This presentation focused on the use of inhibitor peptide leads in order to optimize small molecule drug candidates targeting CDK2/CyclinA.

The rationale behind developing CDK2/CyclinA inhibitors for the treatment of cancer was validated by the fact that during the cell cycle there are a variety of CDK/Cyclin dependent kinases which are critical in order to allow the overall progression to occur (Figure 1). CDK2/CyclinA kinases play a major role in the S phase of the cell cycle. By inhibiting this kinase using peptides the resulting biological cascade affords apoptosis. Through the selective eradication of tumour cells the development of a CDK2/CyclinA small molecule inhibitor would be beneficial as an anti-cancer agent.

**Figure 1**



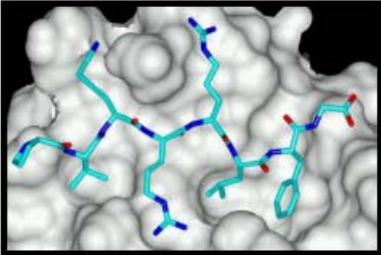
The peptide lead (PEN-LFG) was broken down into two parts, a portion which was necessary for cell penetration, and a portion which was needed for the inhibition of the kinase. Initial assay results also showed this peptide to be selective for killing tumour cells in preference to normal cells.

Medicinal chemistry historically has shown us that there are several problems with the use of peptides in drug discovery (Figure 2). These issues prompted the attempt to convert the original peptide lead to a drug-like small molecule inhibitor.

Figure 2

### Issues with Peptides in Drug Discovery

*Not cell permeable*  
**Rapidly metabolized**  
**Not orally bio-available**

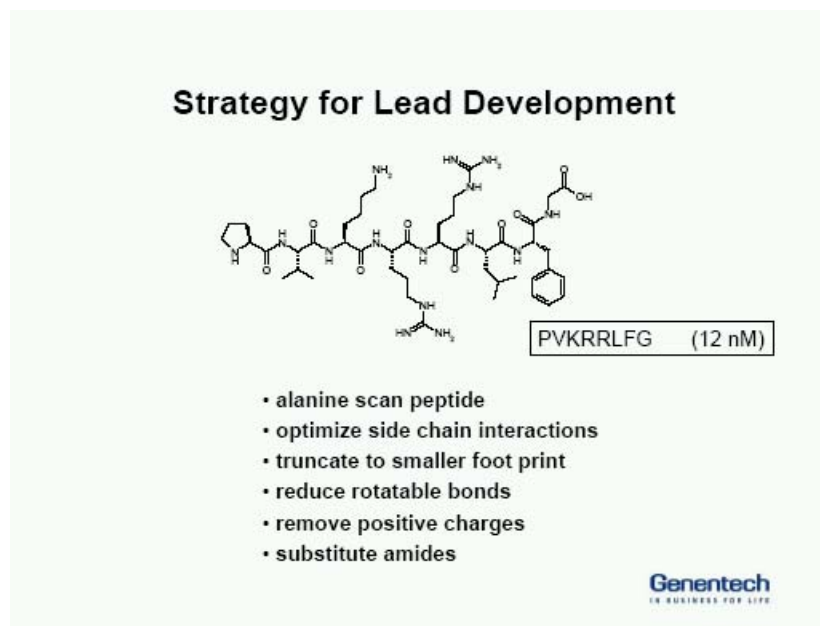


Options for discovering new leads:  
High throughput screening - binding site too shallow  
Literature - nothing reported at the start  
Peptide - challenging but viable

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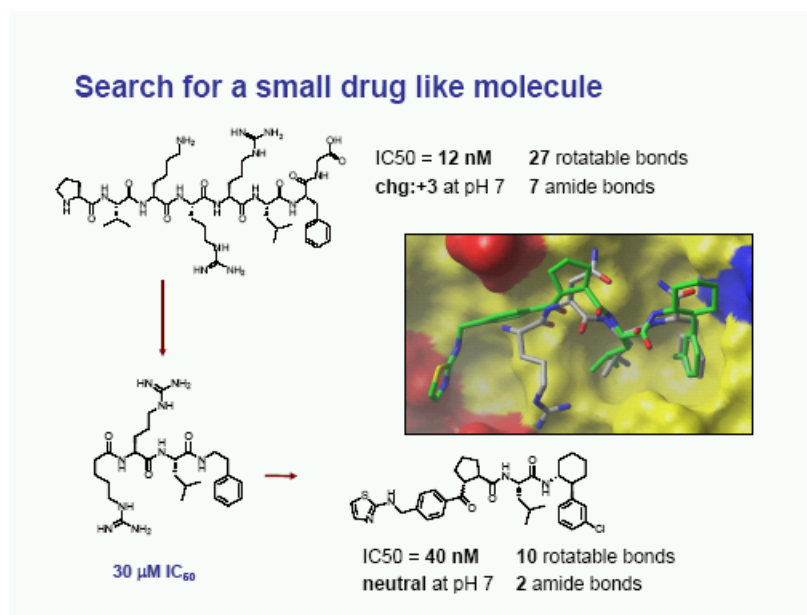
The initial hypothesis was that by developing a small molecule which focused just on the inhibitor portion of the original peptide lead (PVKRRLFG) the researchers could install functionality which would allow this inhibitor to become cell permeable, while retaining the necessary inhibition properties. Through the use of synthetic chemistry (SAR; hundreds of compounds), X-ray crystallographic analysis, and biological assay results the team at Genentech found which residues and functional groups were necessary for binding. Figure 3 illustrates the overall strategy for the lead development of a small molecule inhibitor from the original peptide.

**Figure 3**



As a result of the global SAR using the strategy presented in Figure 3 a small molecule inhibitor was developed which maintained good inhibition characteristics. Additionally, this inhibitor installed drug-like properties which should generate a desirable pK profile (Figure 4).

**Figure 4**



No further pre-clinical data was presented for this inhibitor.