

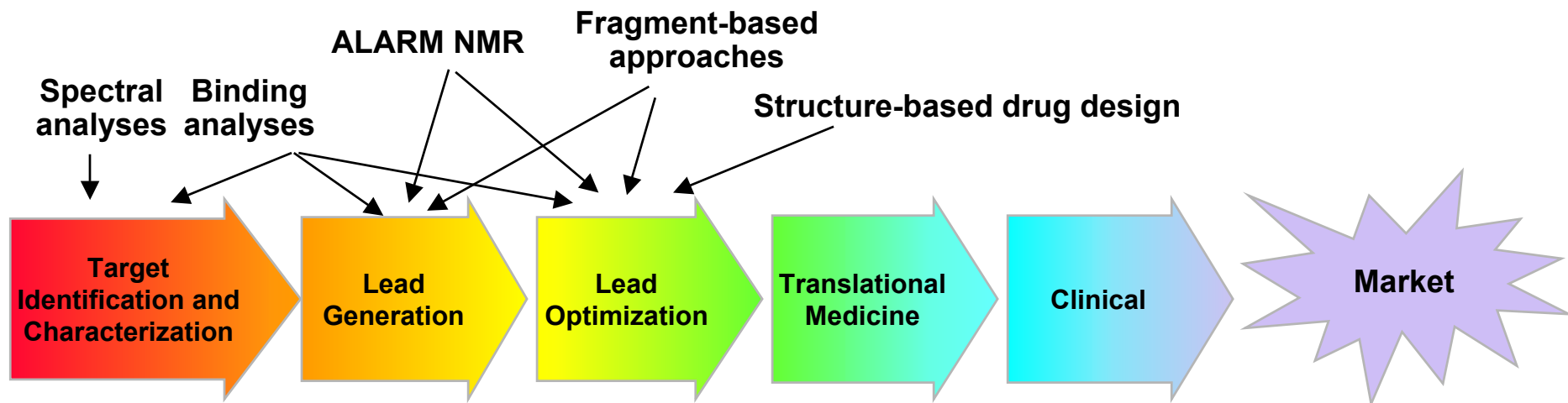


Protein-Ligand Interactions: Lessons from a Decade of NMR-Based Screening

- Philip Hajduk

Protein NMR, Molecular Modeling, and
Cheminformatics

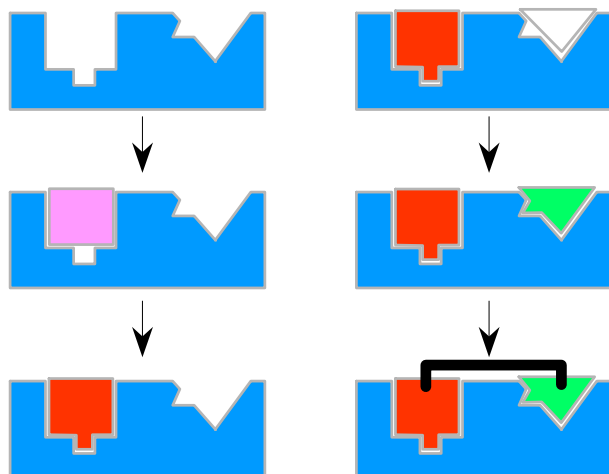
Integration of NMR into the Drug Discovery Pipeline



- Structure-Based Drug Design
- Lead Validation
- Fragment-Based Screening
 - 10,000 cmpds
 - $\langle MW \rangle = 215$

Fragment-Based Drug Design

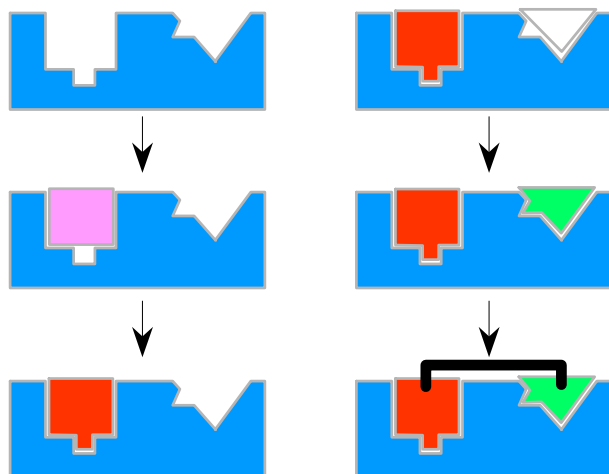
Linked-Fragment



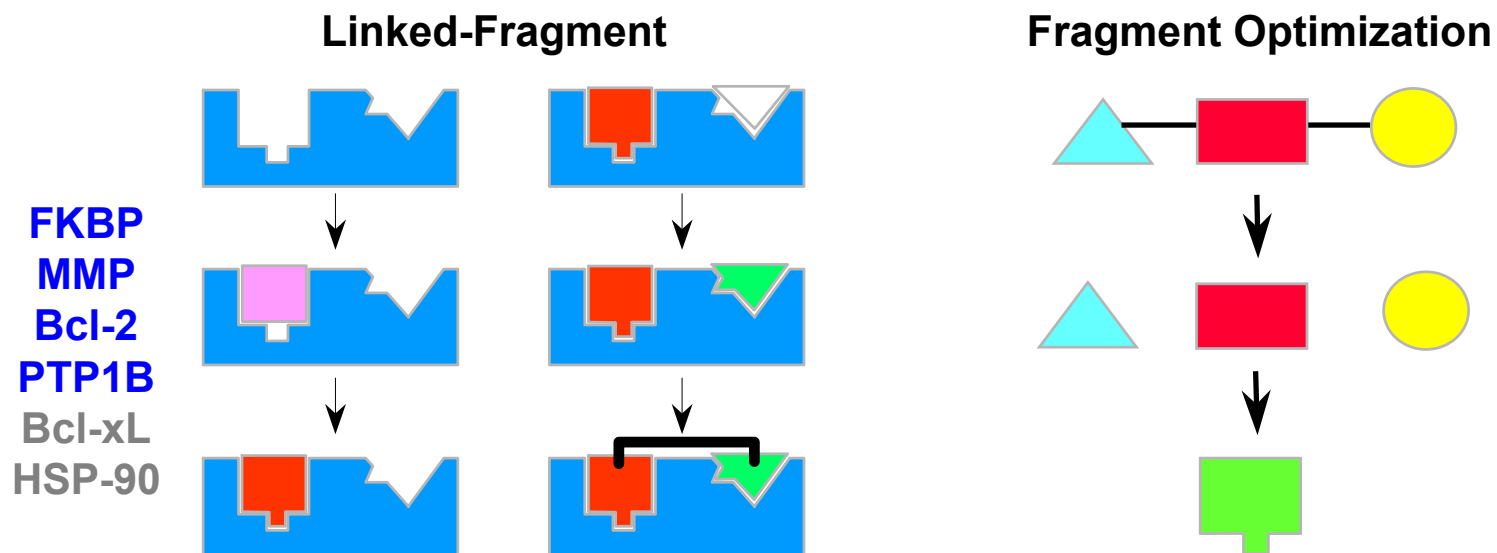
Fragment-Based Drug Design

Linked-Fragment

FKBP
MMP
Bcl-2
PTP1B
Bcl-xL
HSP-90



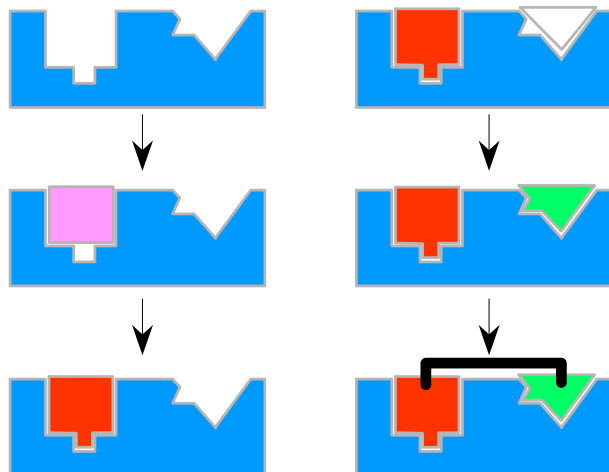
Fragment-Based Drug Design



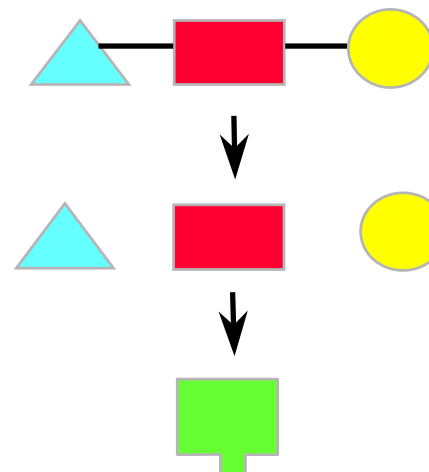
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Fragment Optimization

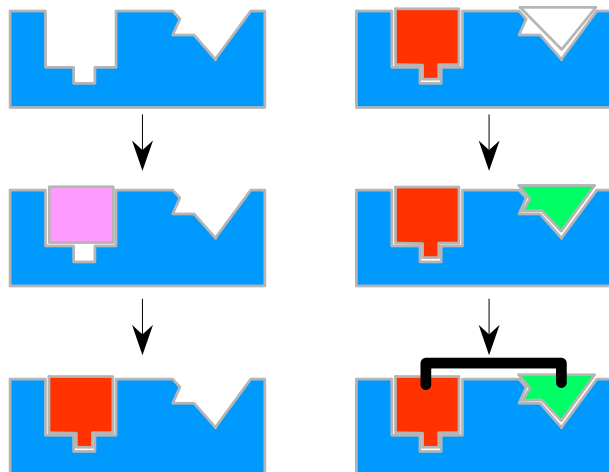


AK
LFA
MMP
MetAP2
PTP1B
Lck-SH2

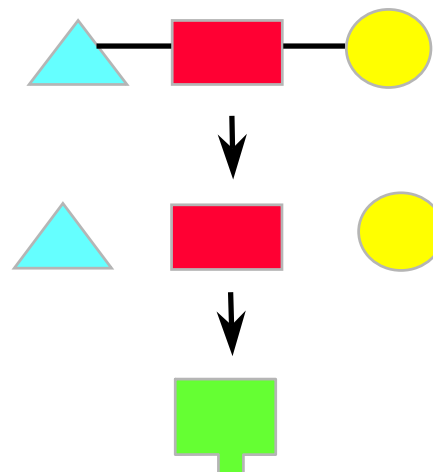
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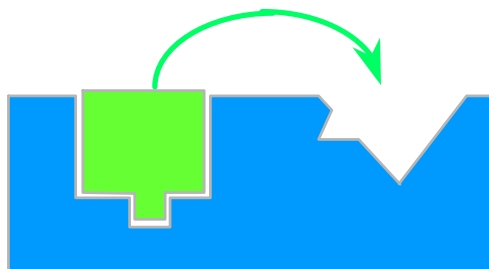


Fragment Optimization



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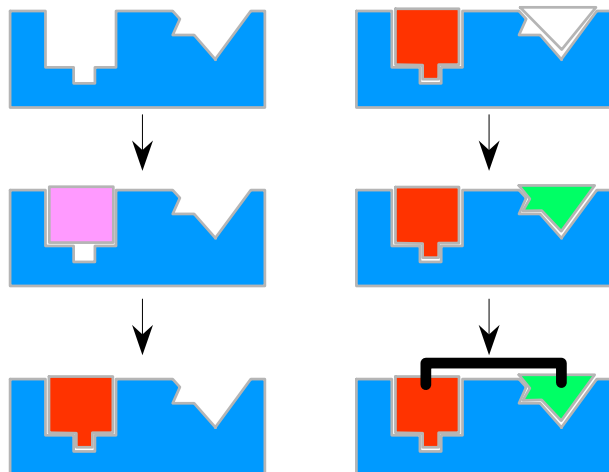
Directed Libraries



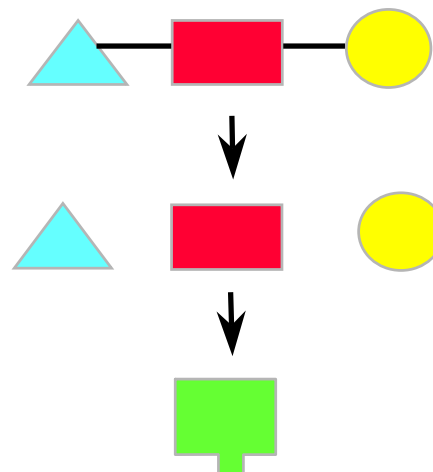
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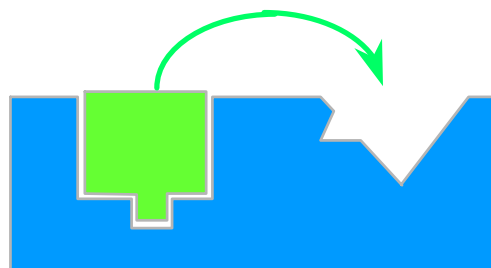


Fragment Optimization



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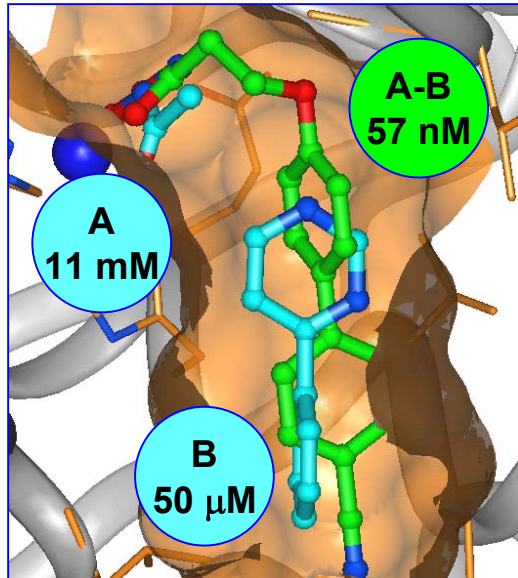
Directed Libraries



Bcl-xL
HSP-90
Survivin
CK2
Akt-1
MDM-2
Mcl-1
Erm-AM

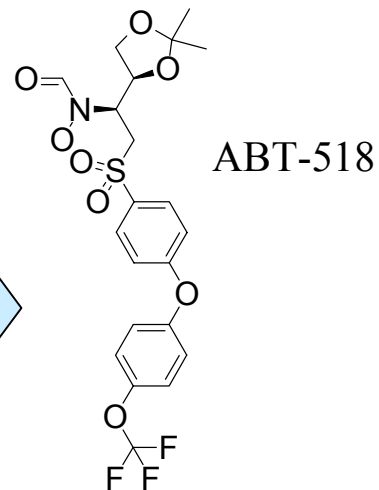
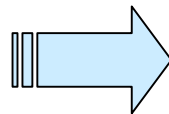
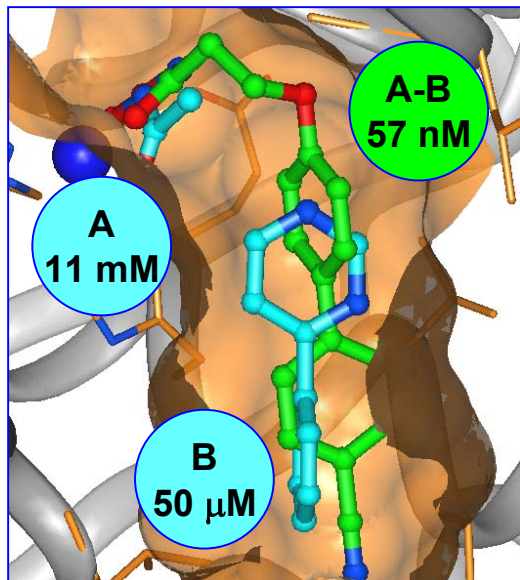
From Fragments to the Clinic

MMP



From Fragments to the Clinic

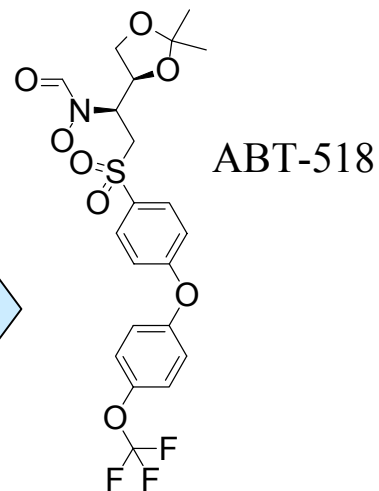
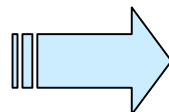
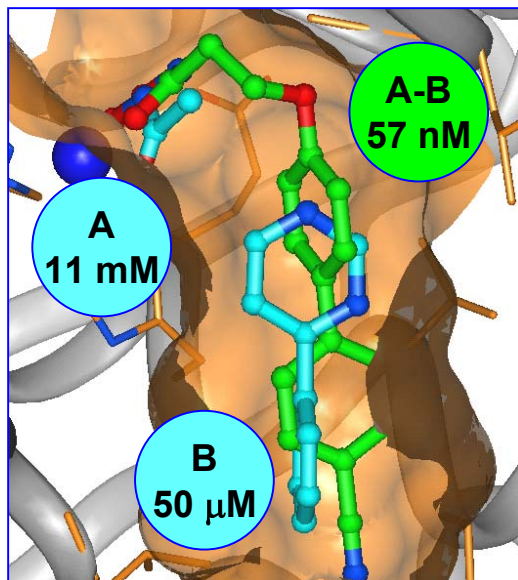
MMP



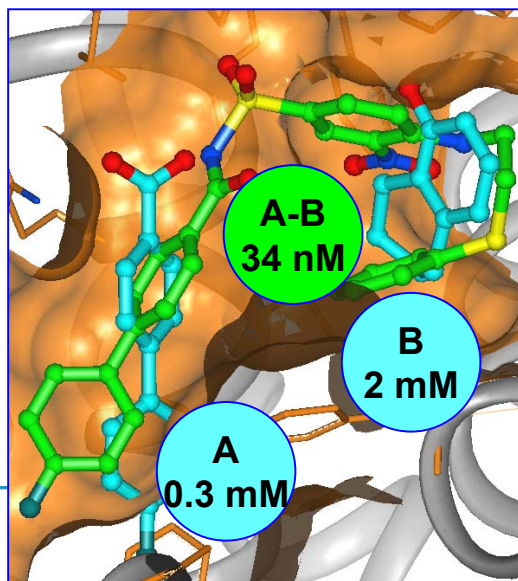
ABT-518

From Fragments to the Clinic

MMP

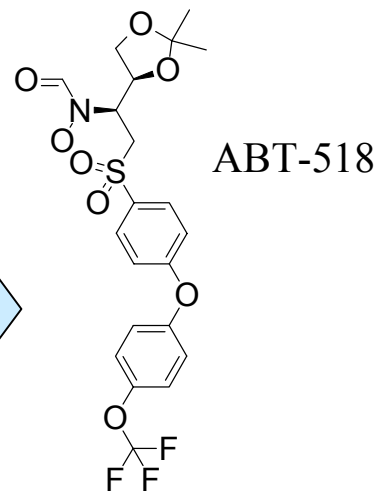
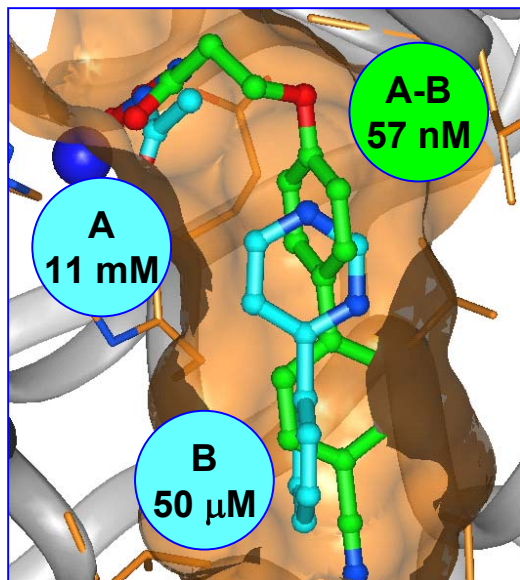


Bcl-2

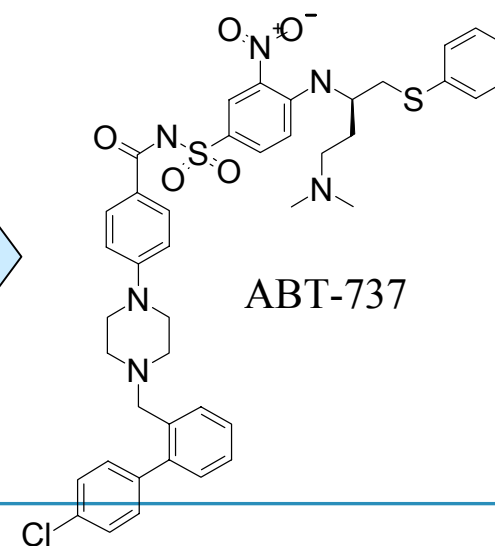
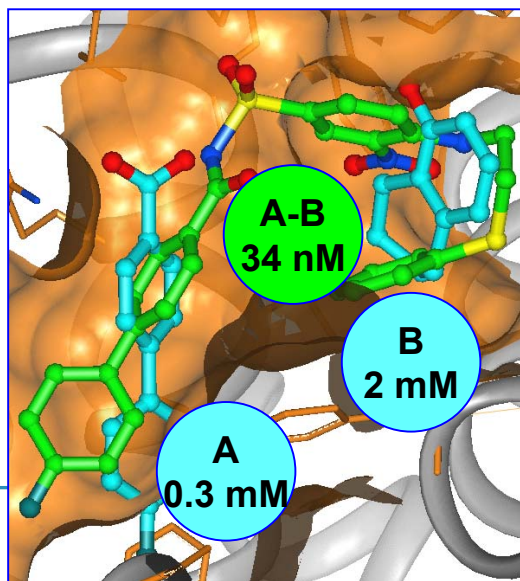


From Fragments to the Clinic

MMP



Bcl-2

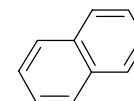
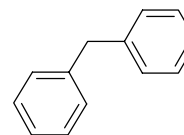
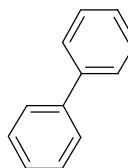


Lessons from a Decade of NMR-Based Screening

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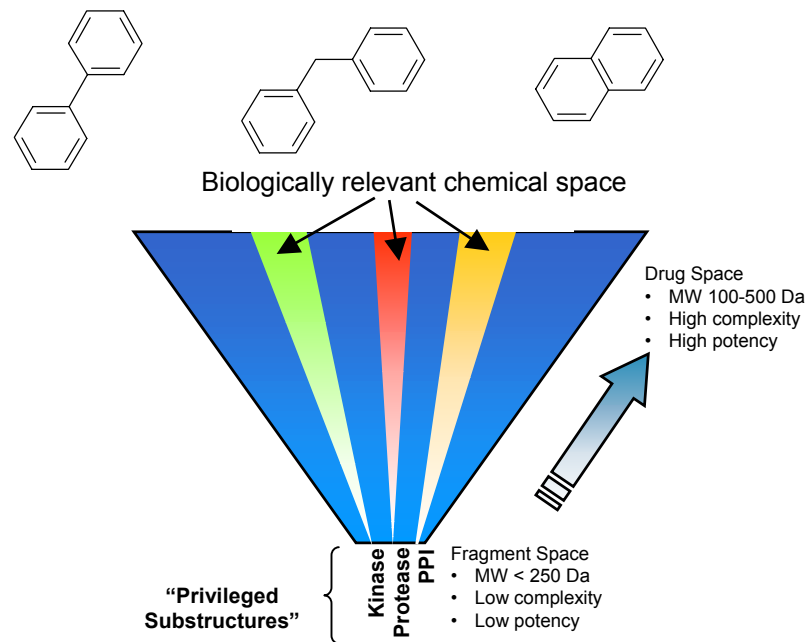
- Privileged structures

- Certain substructures tend to hit with greater frequency
- Hajduk, *et al.* *JMC* 43, 3443-3447 (2000).



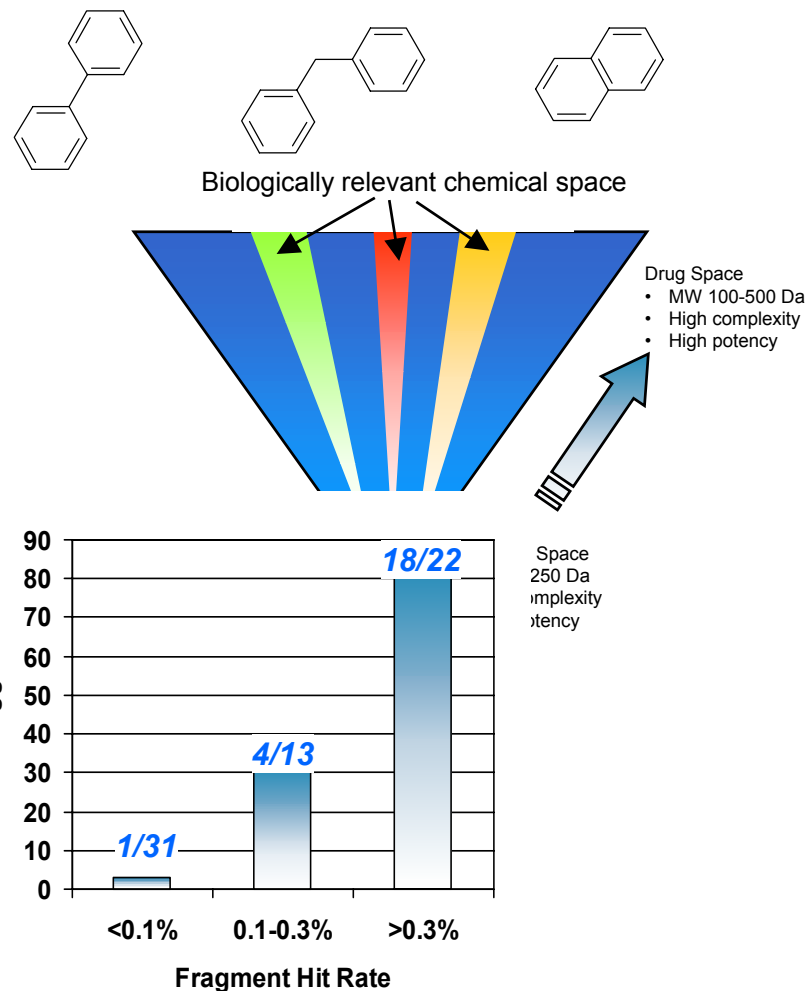
Lessons from a Decade of NMR-Based Screening

- Privileged structures
 - Certain substructures tend to hit with greater frequency
 - Hajduk, *et al.* *JMC* 43, 3443-3447 (2000).
- Biologically relevant chemical space
 - Novel fragment hits can be exploited to fill relevant diversity space
 - Sun, *et al.* *CODDD*. 9, 463-470 (2006).



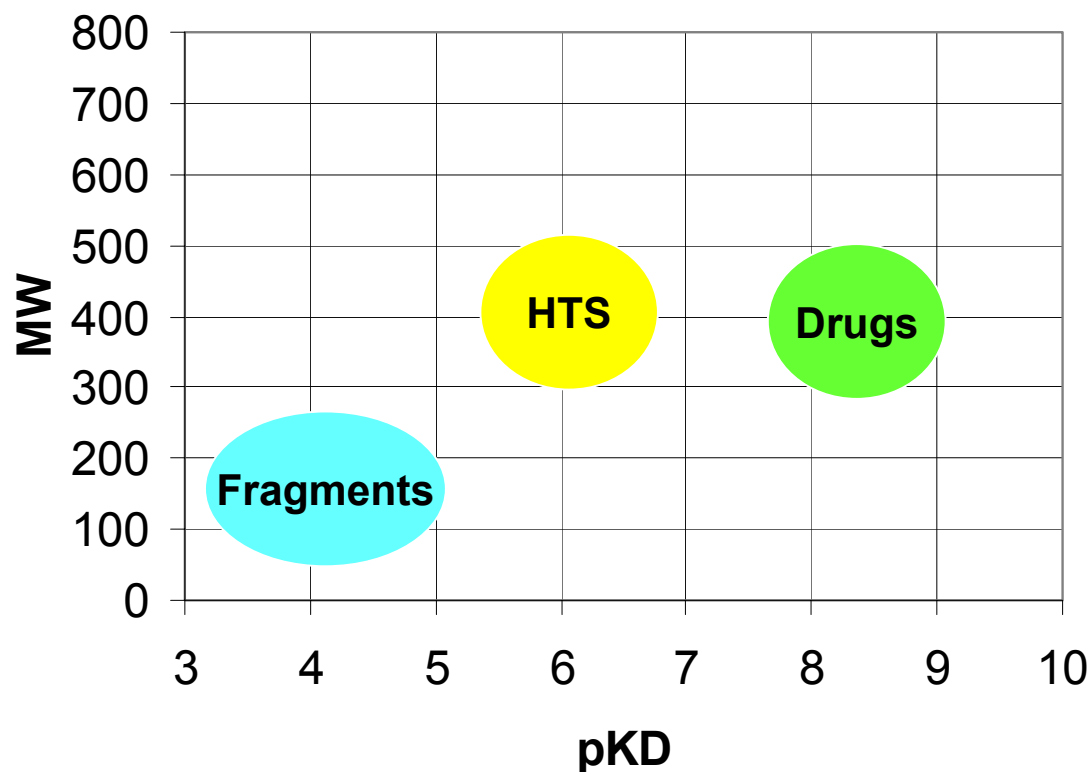
Lessons from a Decade of NMR-Based Screening

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- Biologically relevant chemical space
 - Novel fragment hits can be exploited to fill relevant diversity space
 - Sun, *et al. CODDD*. 9, 463-470 (2006).
- Protein druggability
 - Fragment hit rates are excellent predictors of discovery success
 - Hajduk, *et al. JMC* 48, 2518-2525 (2005)



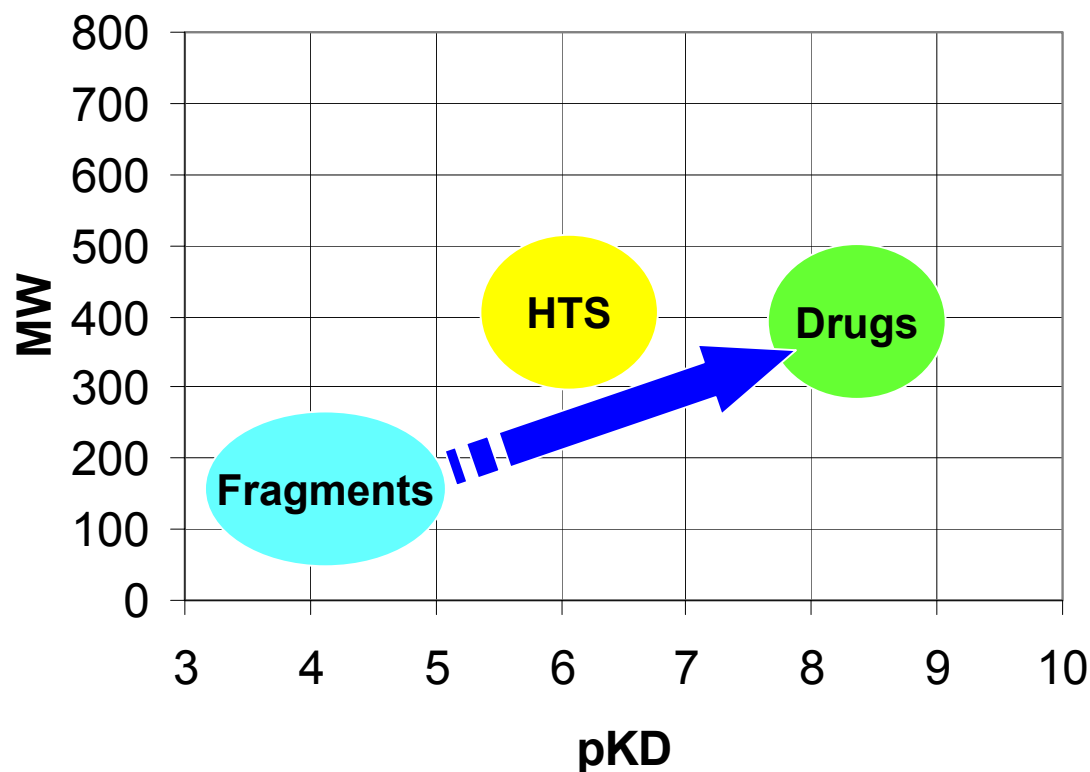
Fragment Selection and Optimization

- Simple view: balance of potency and size



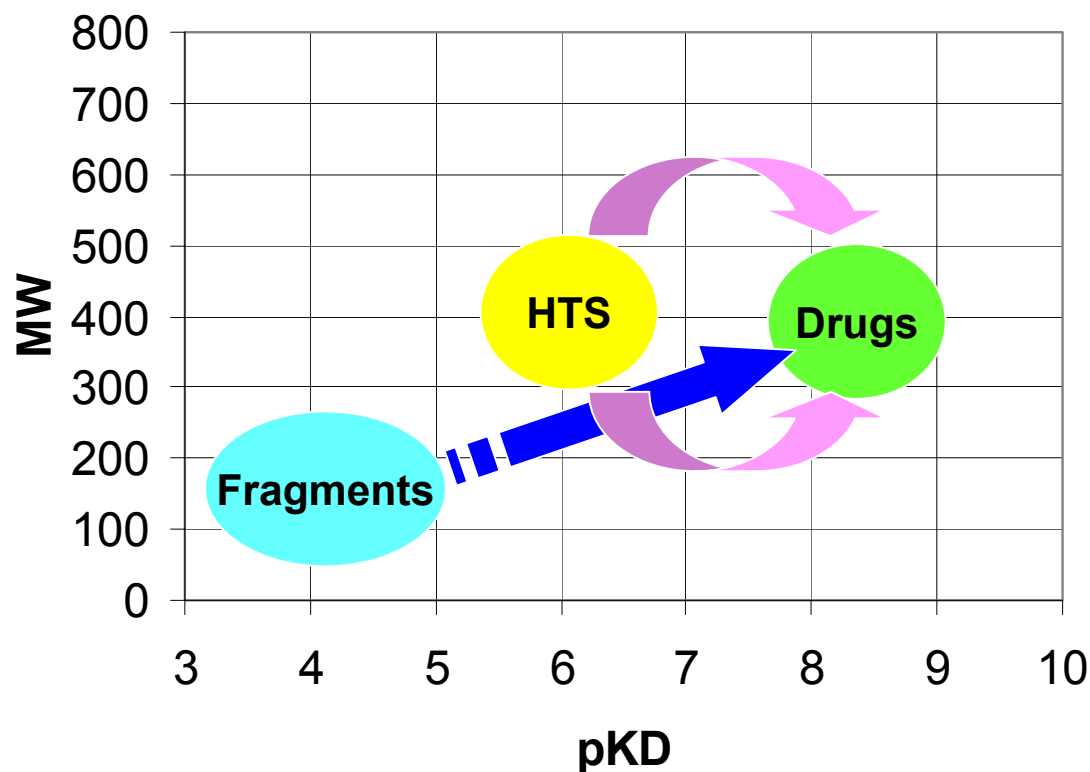
Fragment Selection and Optimization

- Simple view: balance of potency and size
 - Fragment hits: Increase both potency and size



Fragment Selection and Optimization

- Simple view: balance of potency and size
 - Fragment hits: Increase both potency and size
 - HTS hits: variable routes



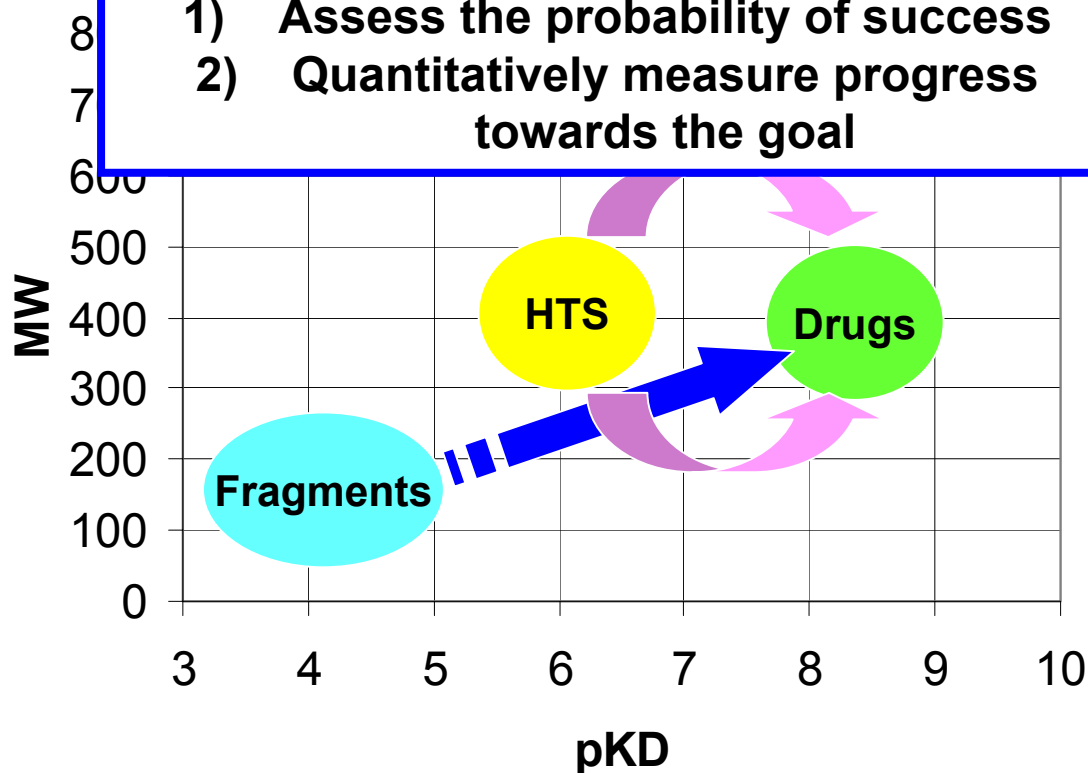
Fragment Selection and Optimization

- Simple view: balance of potency and size

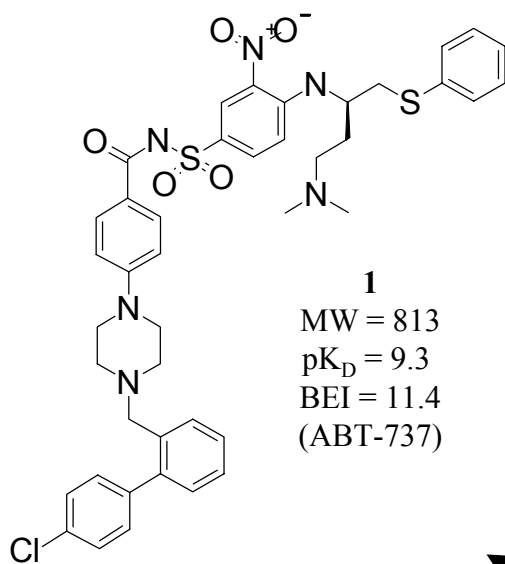
- Fragments
- HTS hits

Goal: Develop rigorous statistical criteria by which we can

- 1) Assess the probability of success**
- 2) Quantitatively measure progress towards the goal**

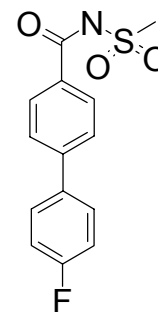


ABT-737 (Bcl-xL) Candidate Deconstruction



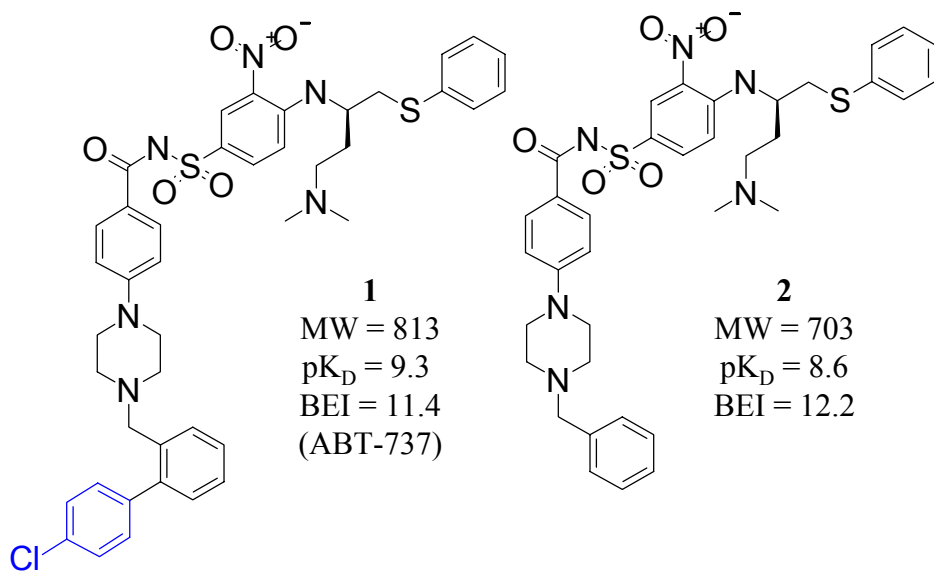
1
MW = 813
pK_D = 9.3
BEI = 11.4
(ABT-737)

Fragment Lead

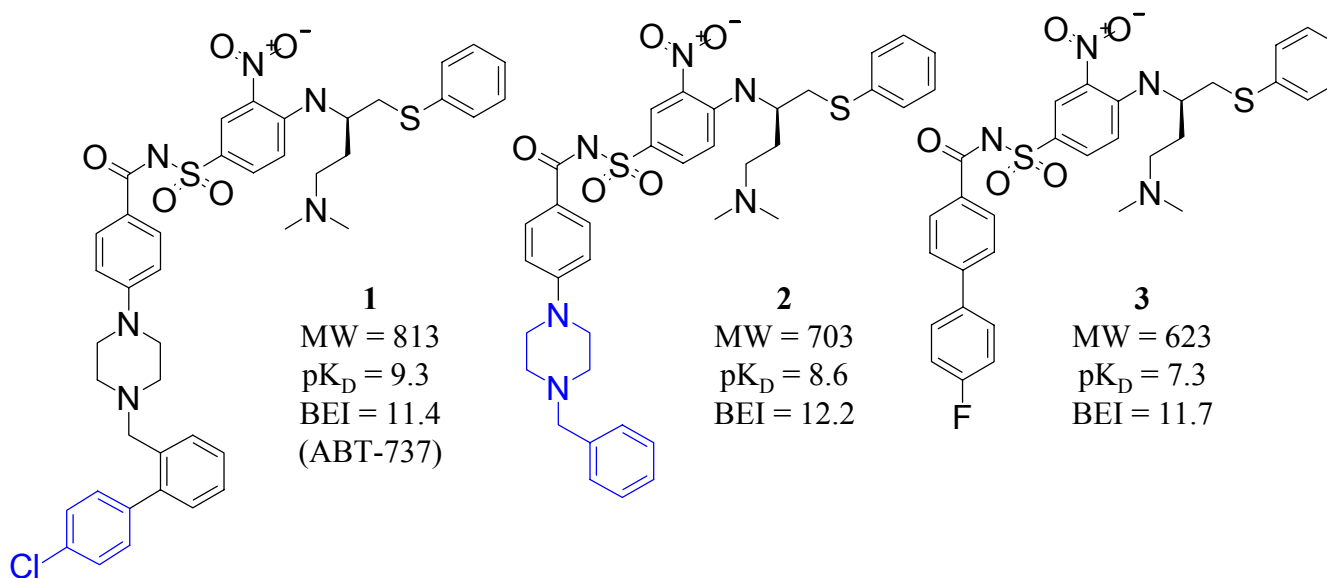


6
MW = 293
pK_D = 3.5
BEI = 11.9

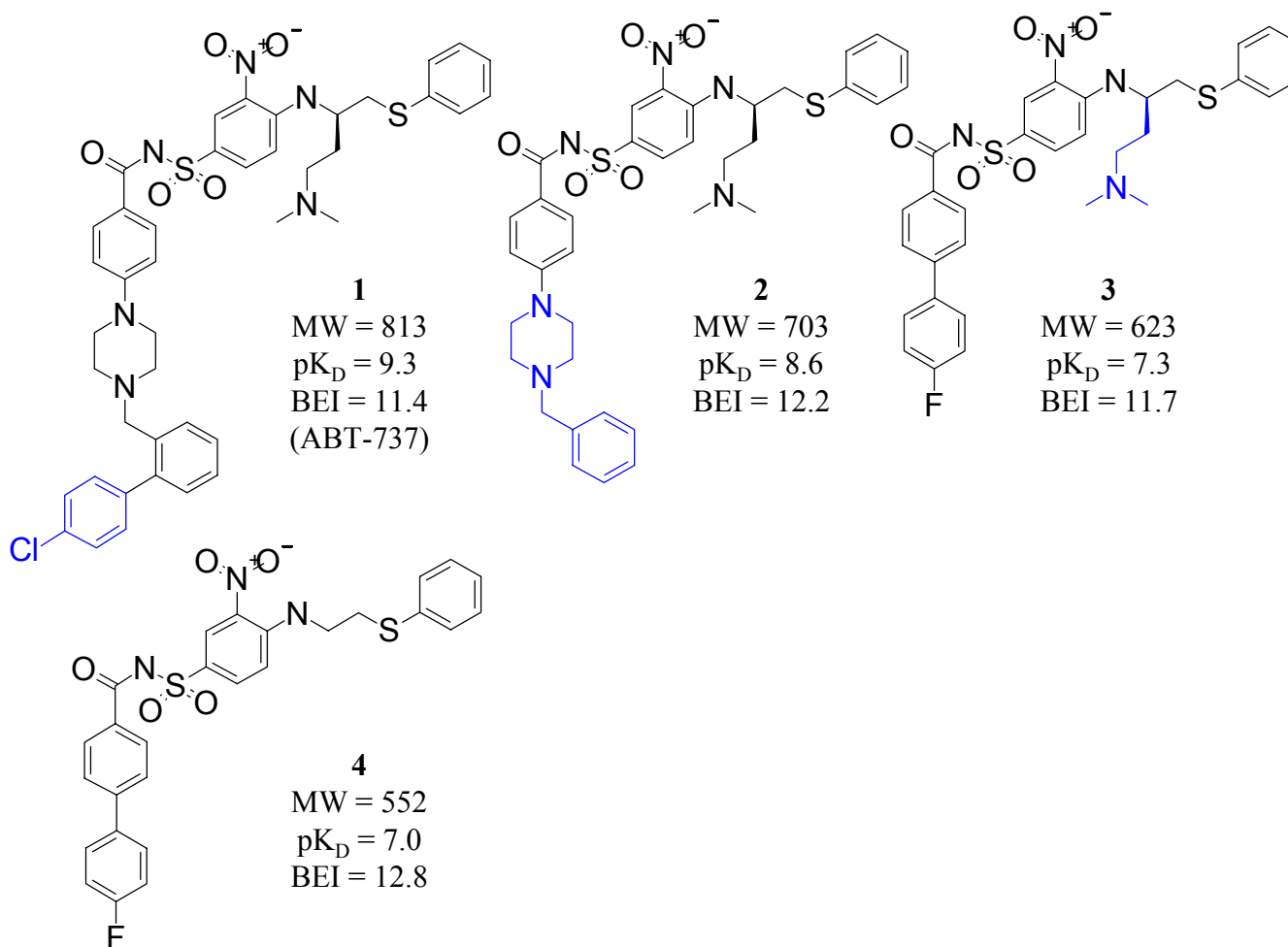
ABT-737 (Bcl-xL) Candidate Deconstruction



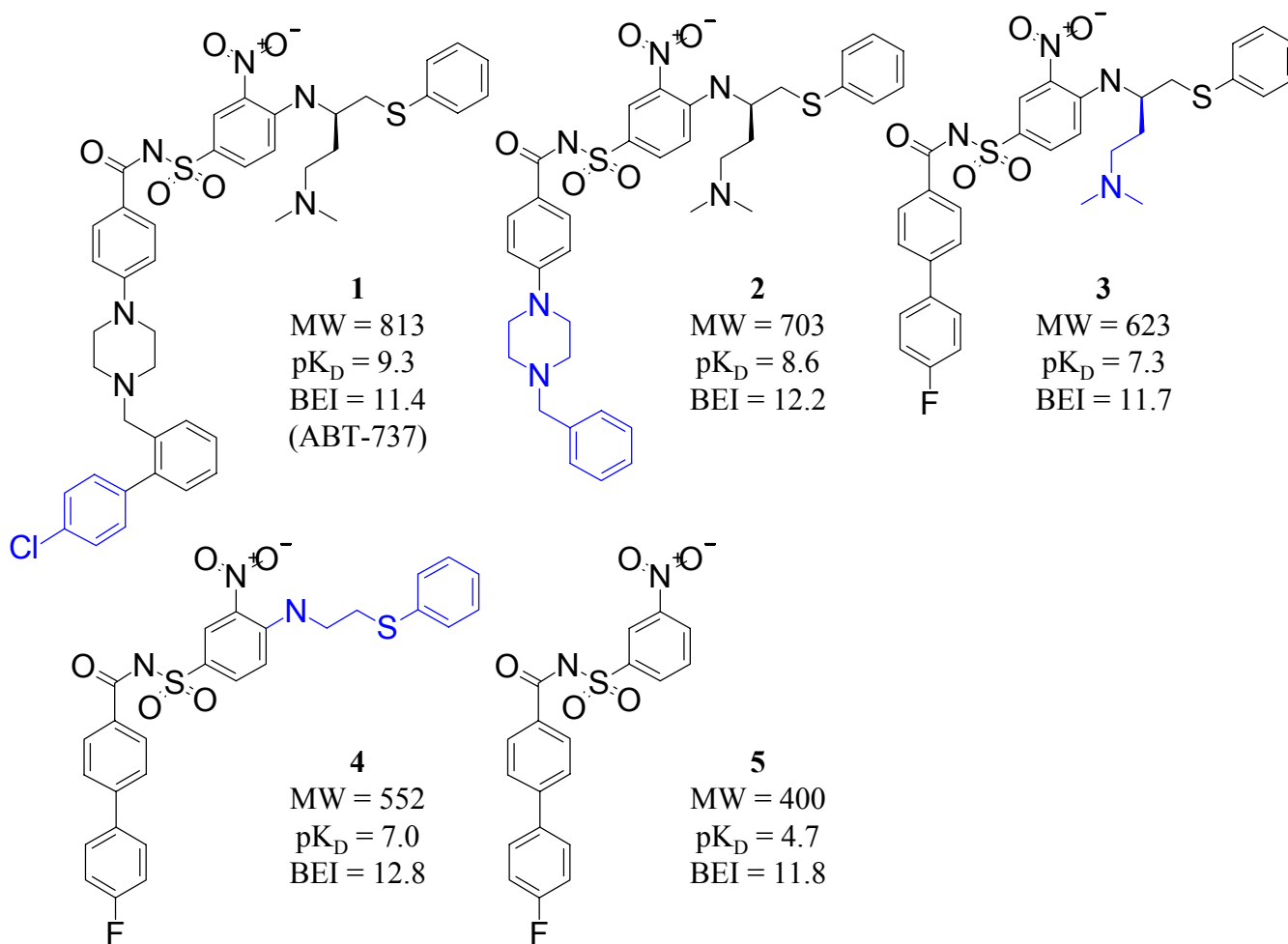
ABT-737 (Bcl-xL) Candidate Deconstruction



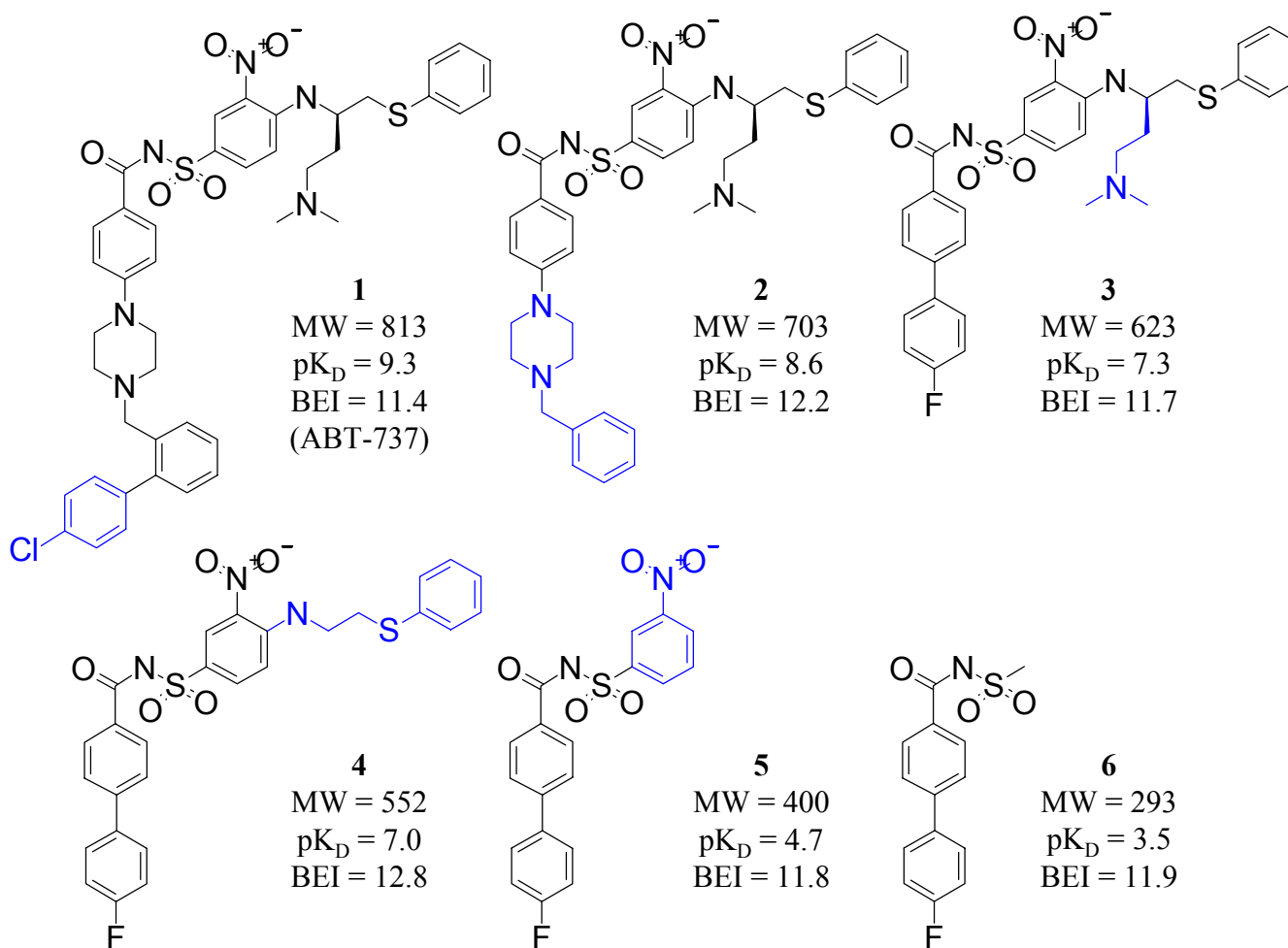
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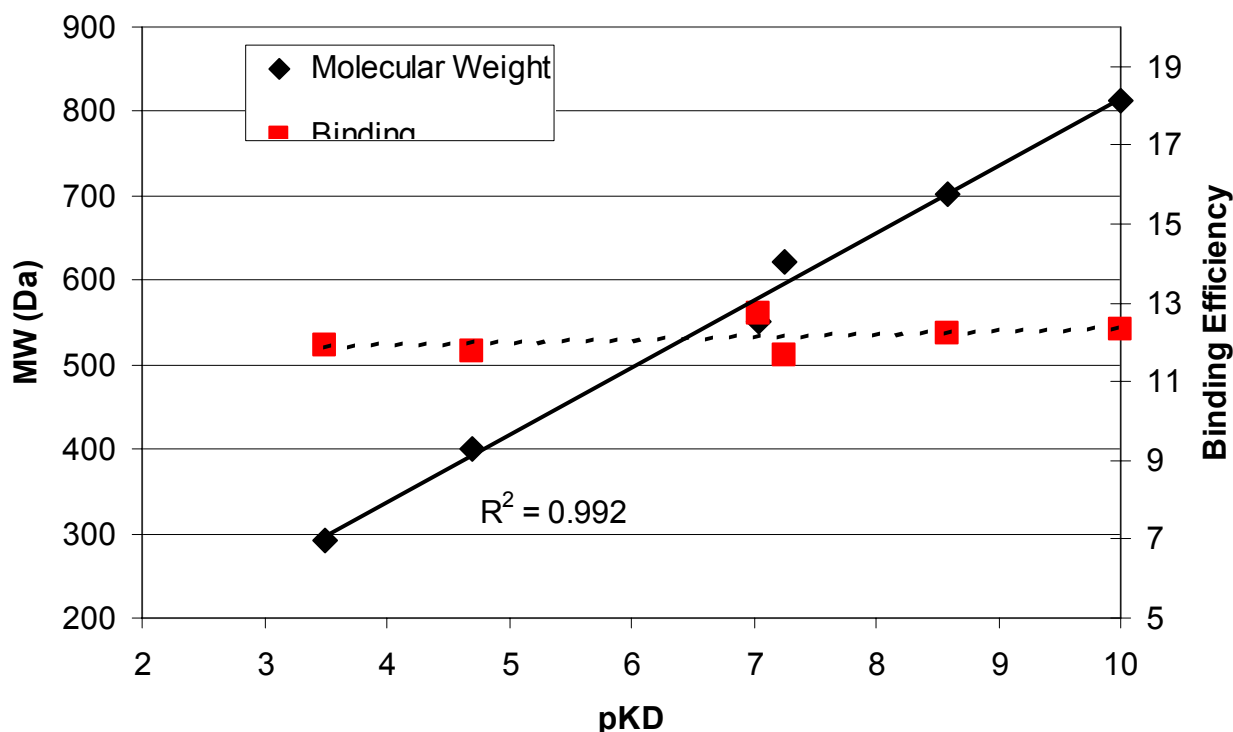


ABT-737 (Bcl-xL) Candidate Deconstruction



ABT-737 (Bcl-xL) Candidate Deconstruction

- pKD and MW almost perfectly linear
- Binding efficiency essentially constant

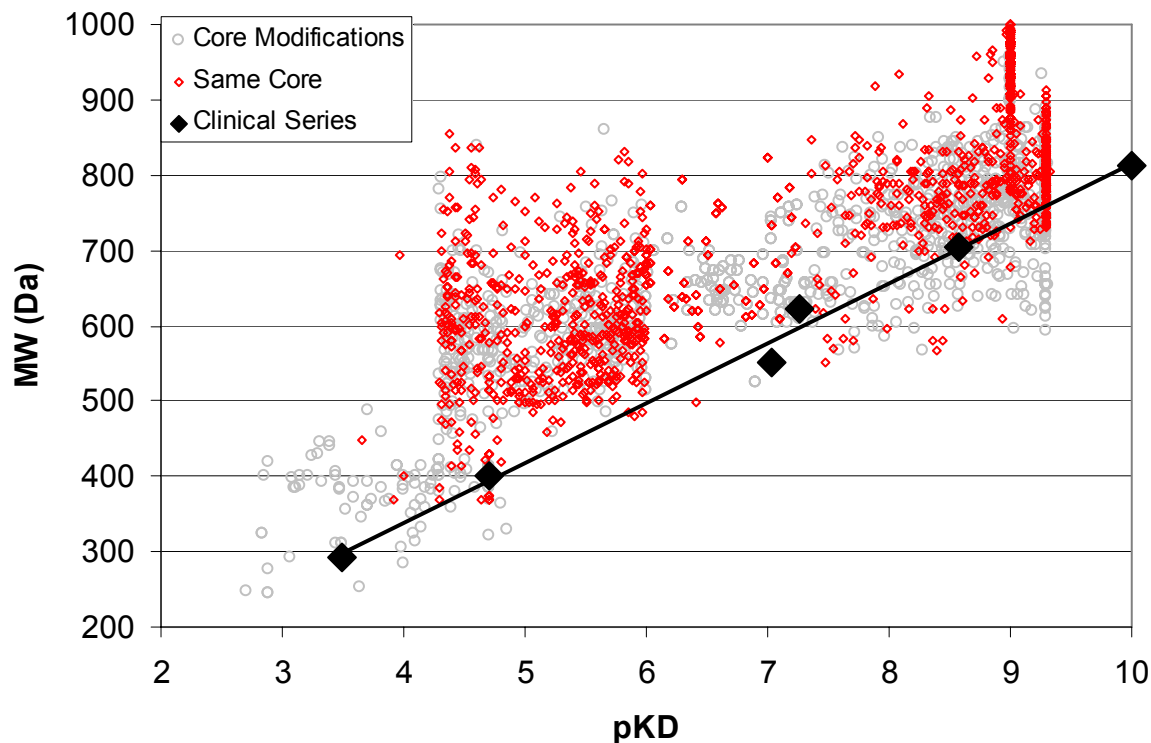


$pK_D = -\log(K_D)$, using K_D , K_i , and IC_{50} interchangeably

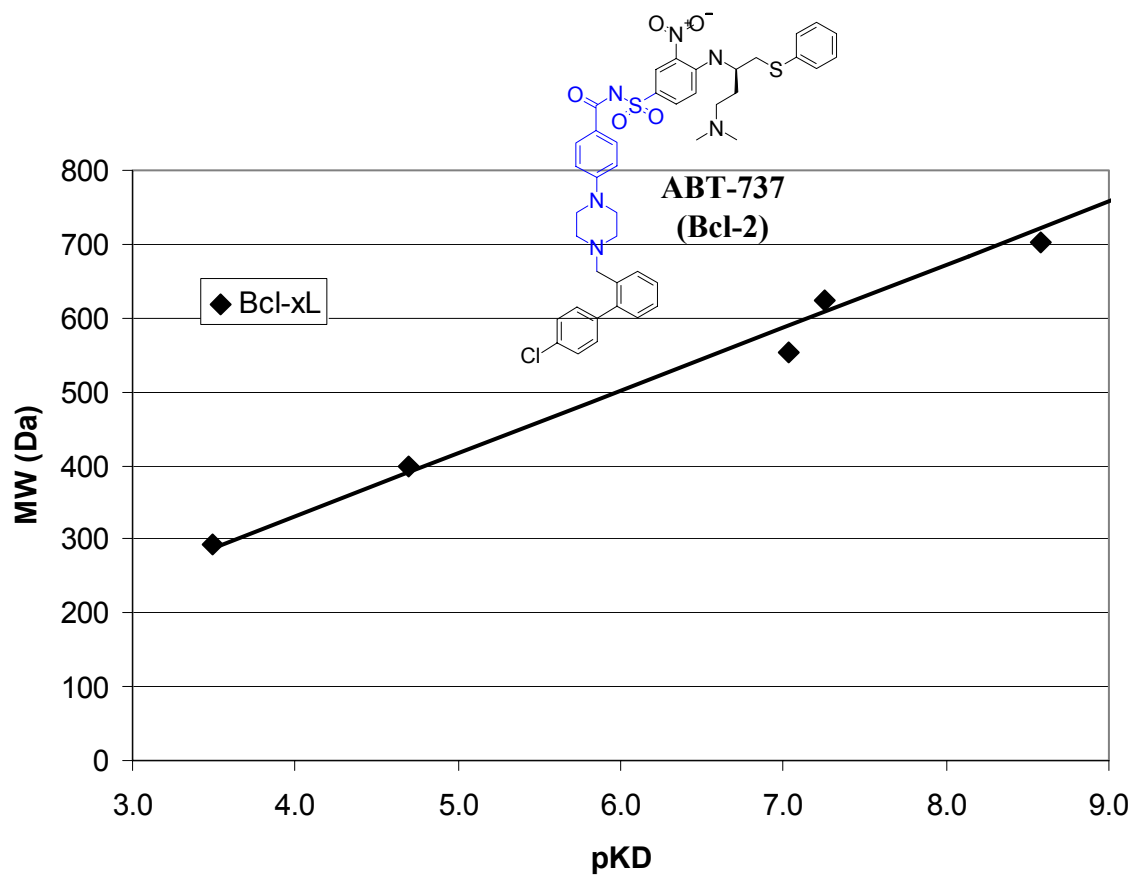
$BEI = pK_D / MW(kDa)$ (r.e. Metz and Abad-Zapatero)

ABT-737 (Bcl-xL): All Compounds in Series

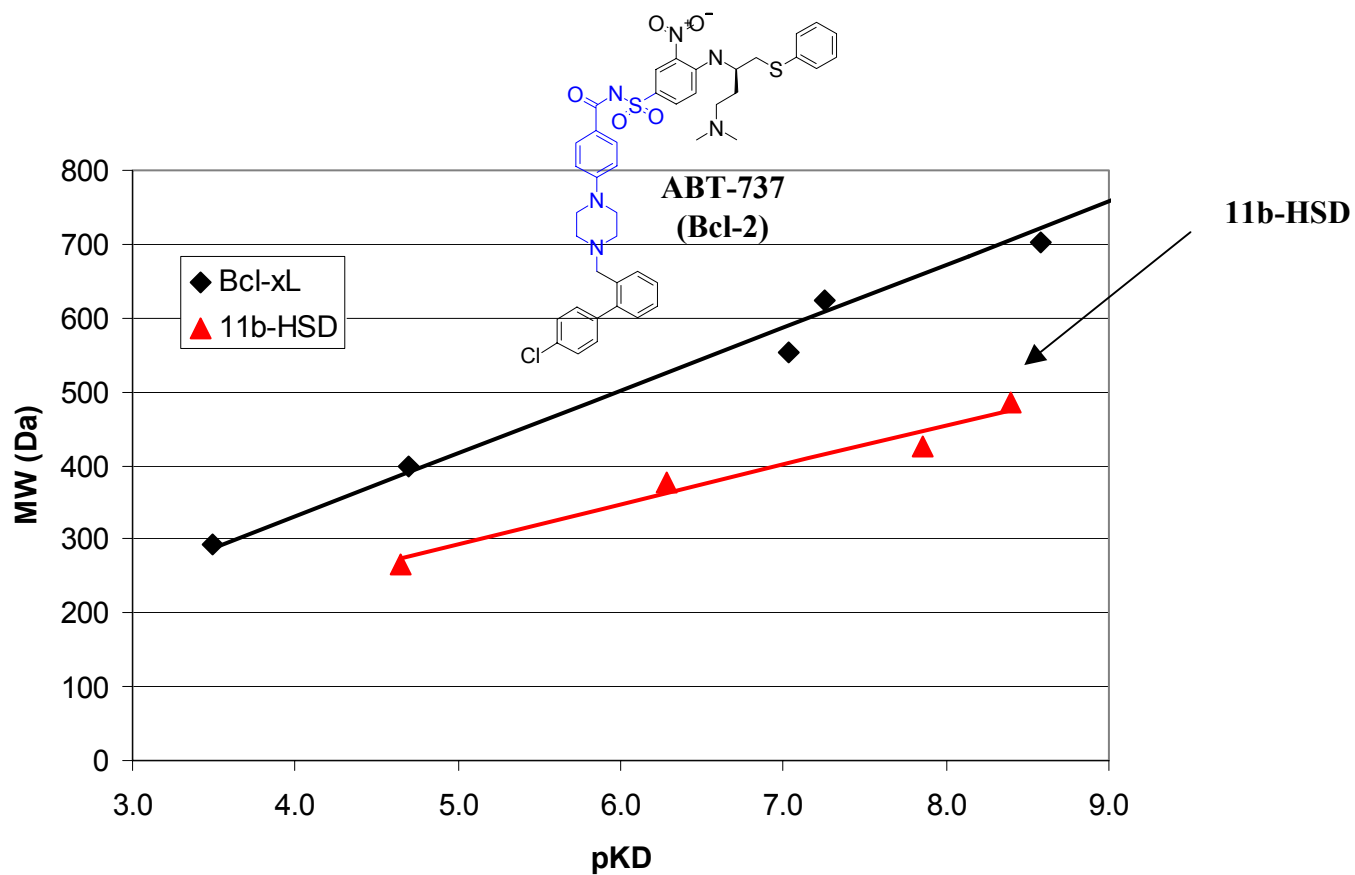
- Clinical series stays at the “front” of the distribution (i.e., most potency per mass)
- Significant number of deviations only when core modifications are involved



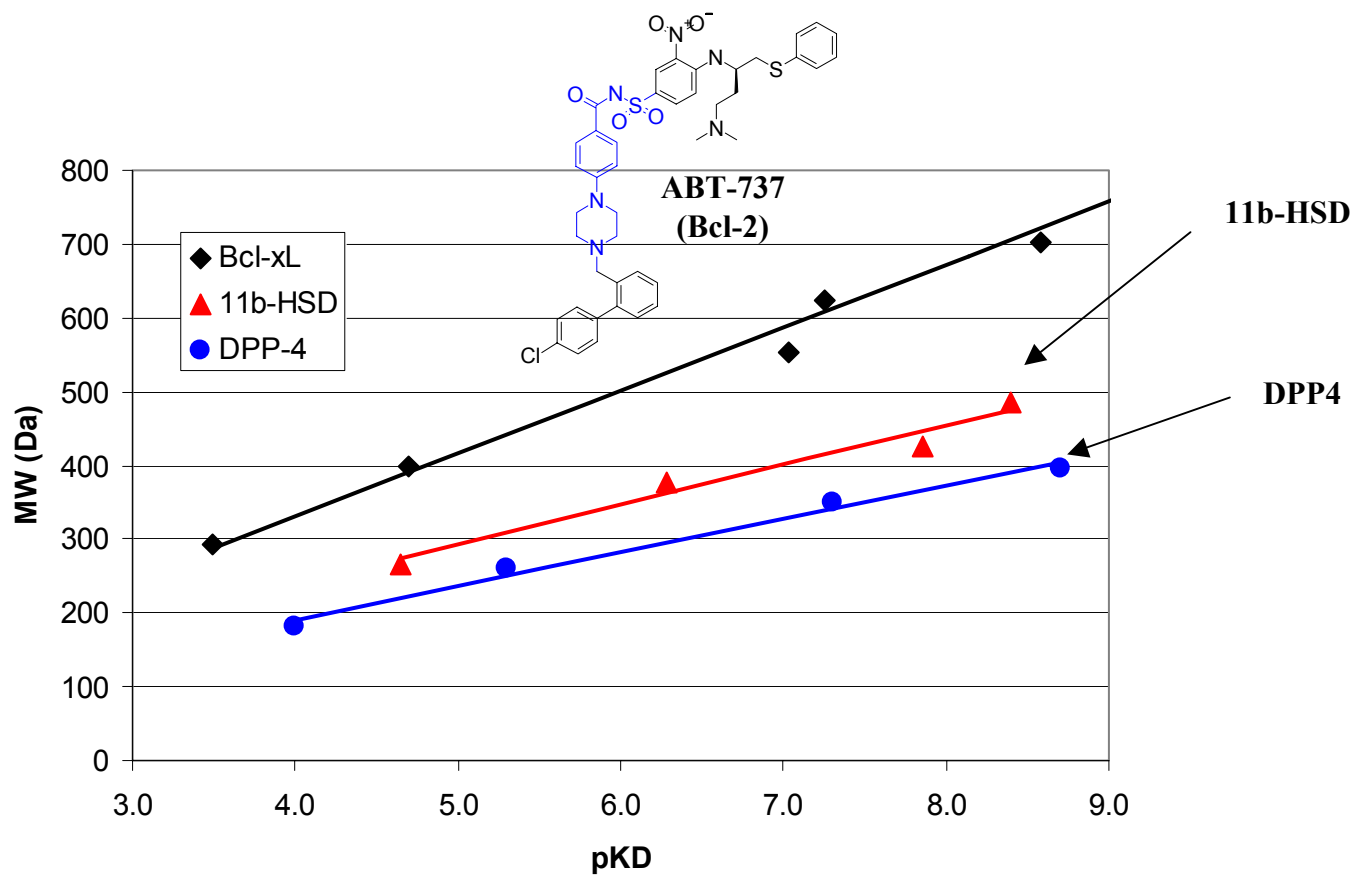
Candidate Deconstruction – Additional Series



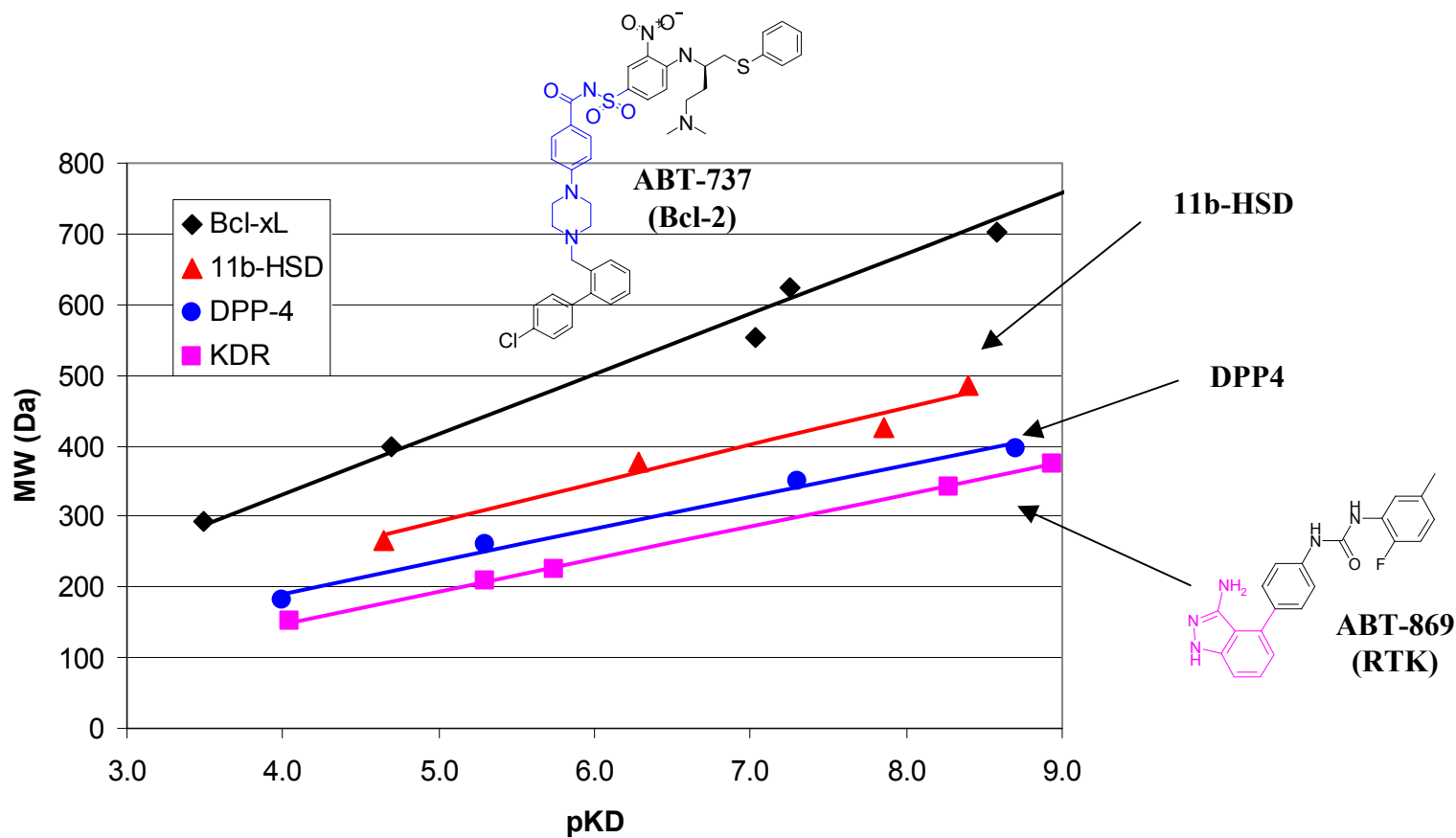
Candidate Deconstruction – Additional Series



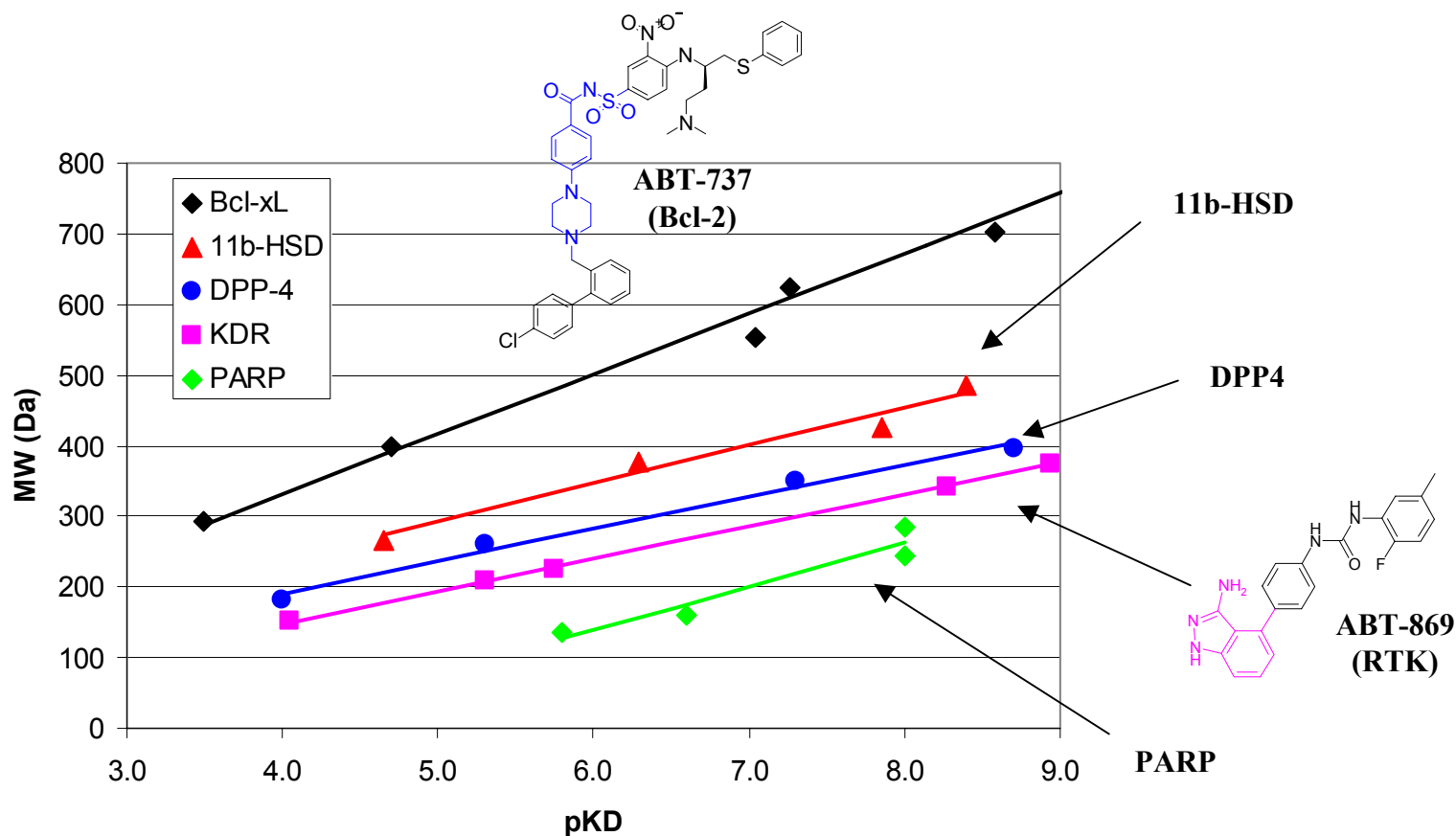
Candidate Deconstruction – Additional Series



Candidate Deconstruction – Additional Series



Candidate Deconstruction – Additional Series



Candidate Deconstruction – All Series

- DDC compounds

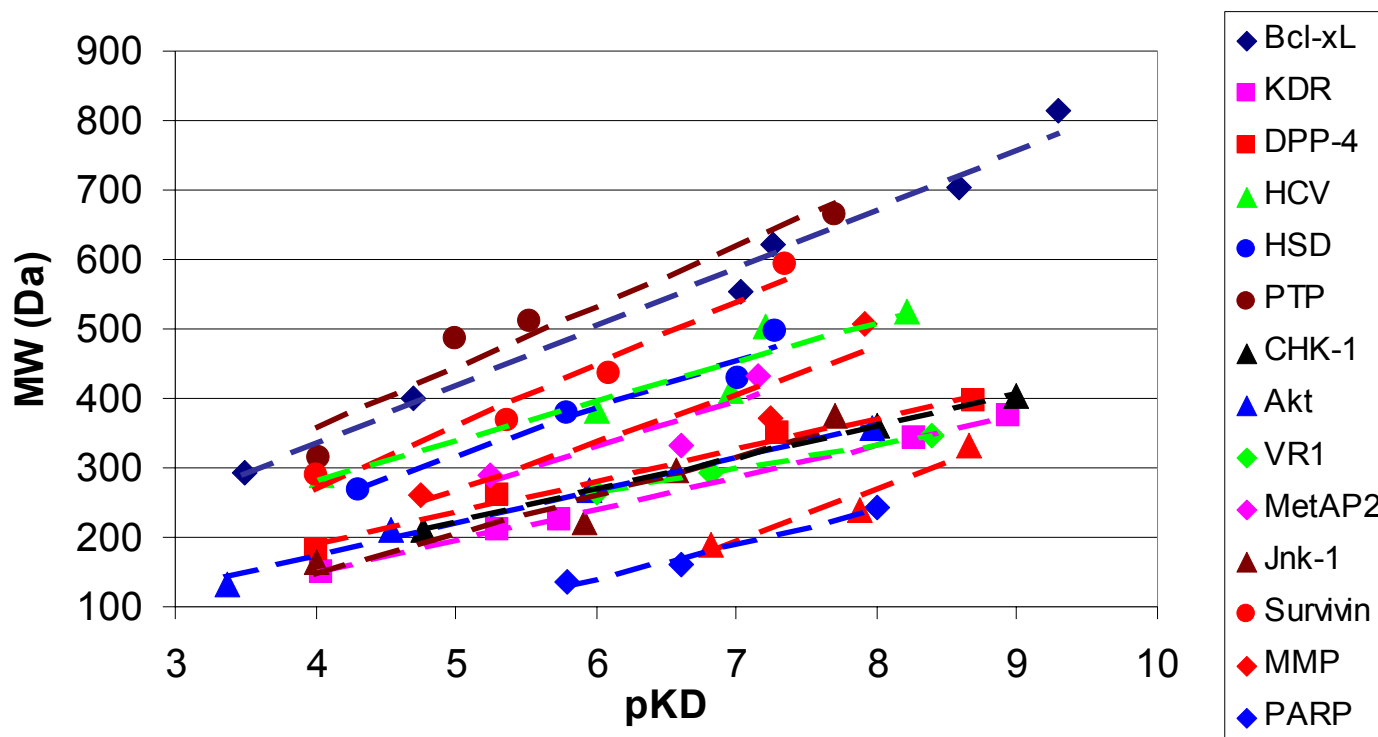
- Bcl-xL
- KDR
- DPP4
- PARP
- VR1
- MMP
- H3

- Highly optimized compounds

- 11 β -HSD
- HCV polymerase
- Akt-1
- CHK-1
- Jnk-1
- MetAP2
- PTP-1B
- Survivin

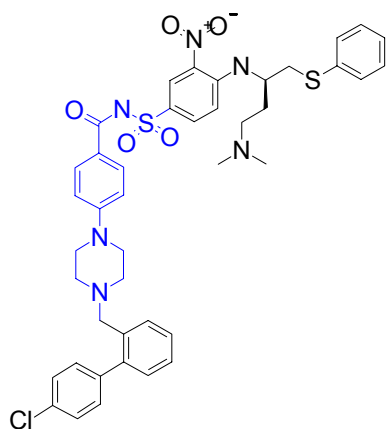
Candidate Deconstruction – All Series

- All deconstructions are linear
- All series are co-linear
 - Average slope of 64 Da/pK_D unit



Implications for Protein-Ligand Interactions

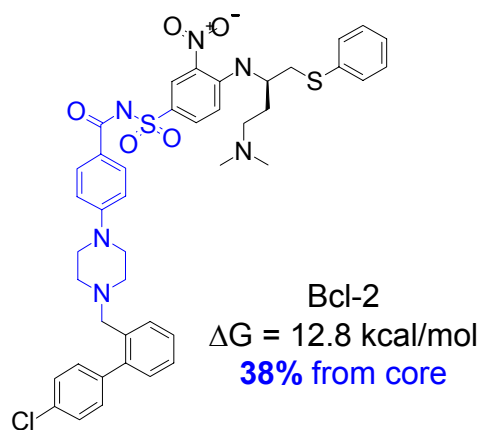
- Compound view
 - Starting from an optimized fragment and following the ideal path for optimization, every mass unit added to the compound contributes positively and equally to the binding affinity.
 - It is the binding energy of the core that differentiates one series from another



Implications for Protein-Ligand Interactions

- Compound view

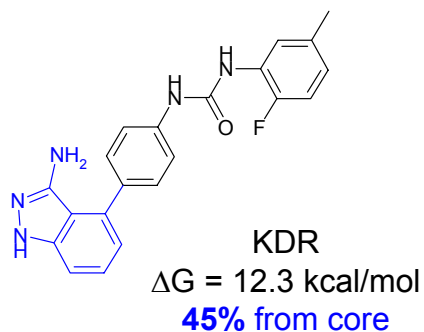
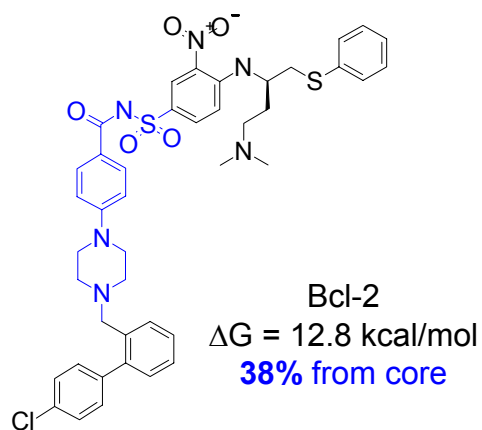
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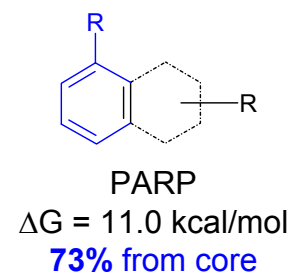
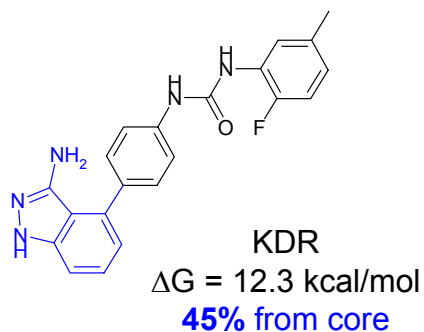
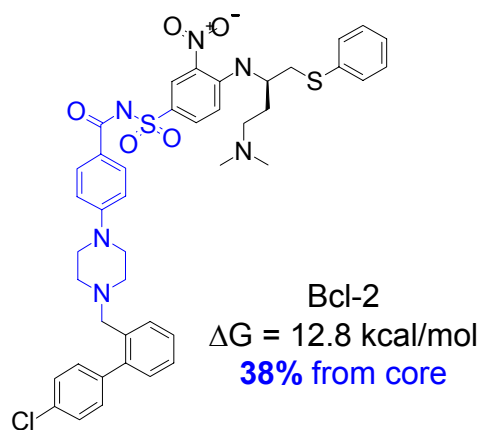
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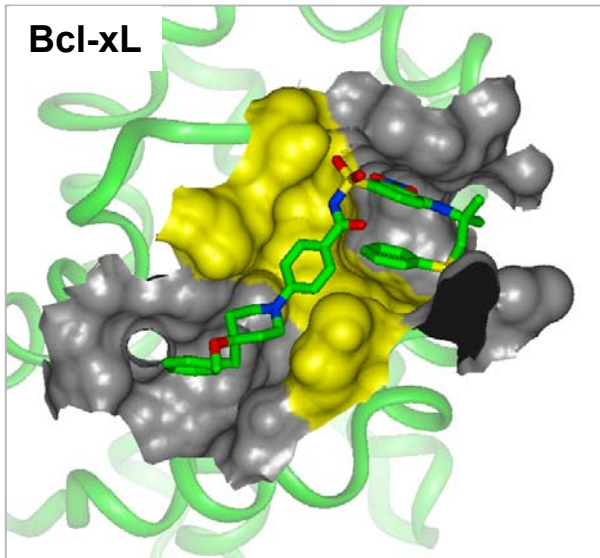
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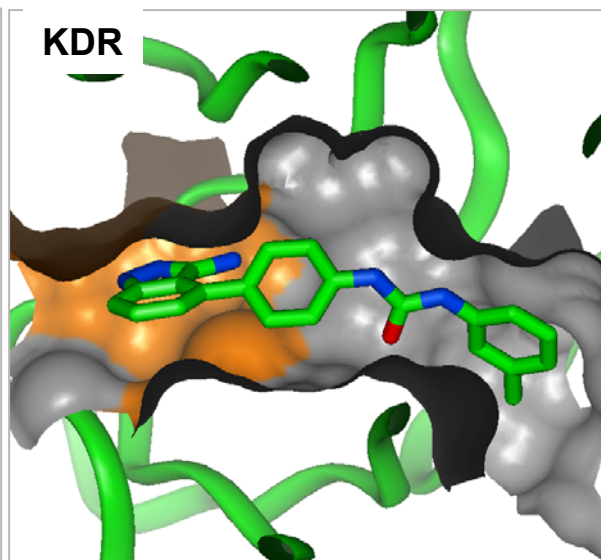
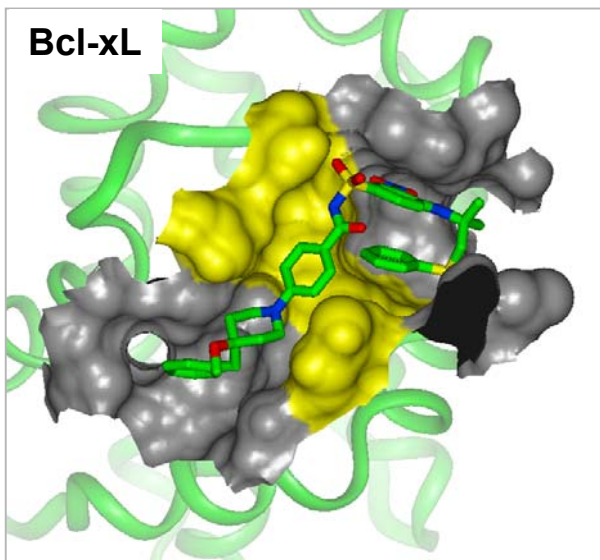
Implications for Protein-Ligand Interactions

- Protein view
 - Starting from the binding hot spot, every \AA^2 involved in the binding interface contributes positively and equally to the binding affinity.
 - It is the binding energy that can be achieved in the hot spot that differentiates one target from another



Implications for Protein-Ligand Interactions

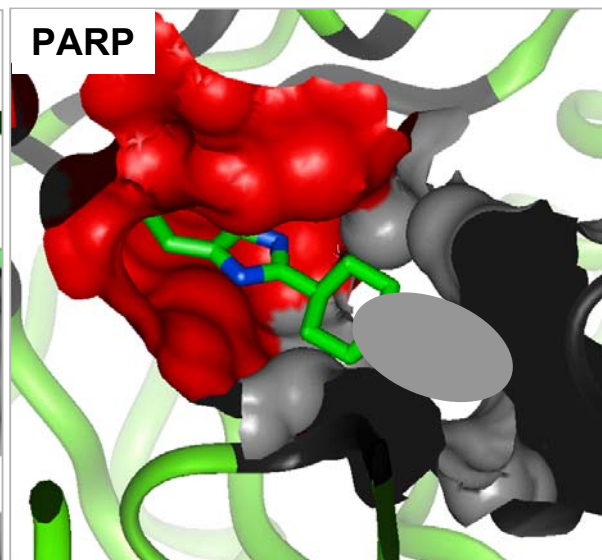
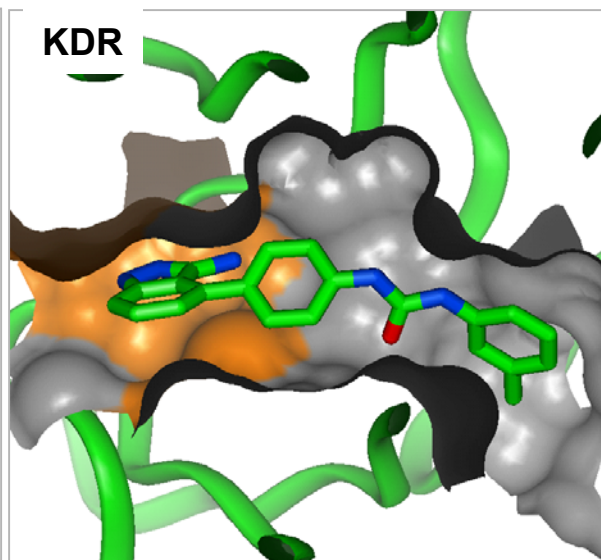
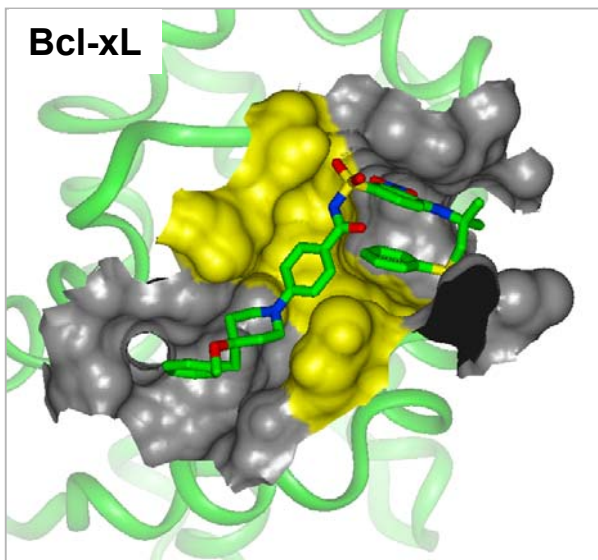
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Implications for Protein-Ligand Interactions

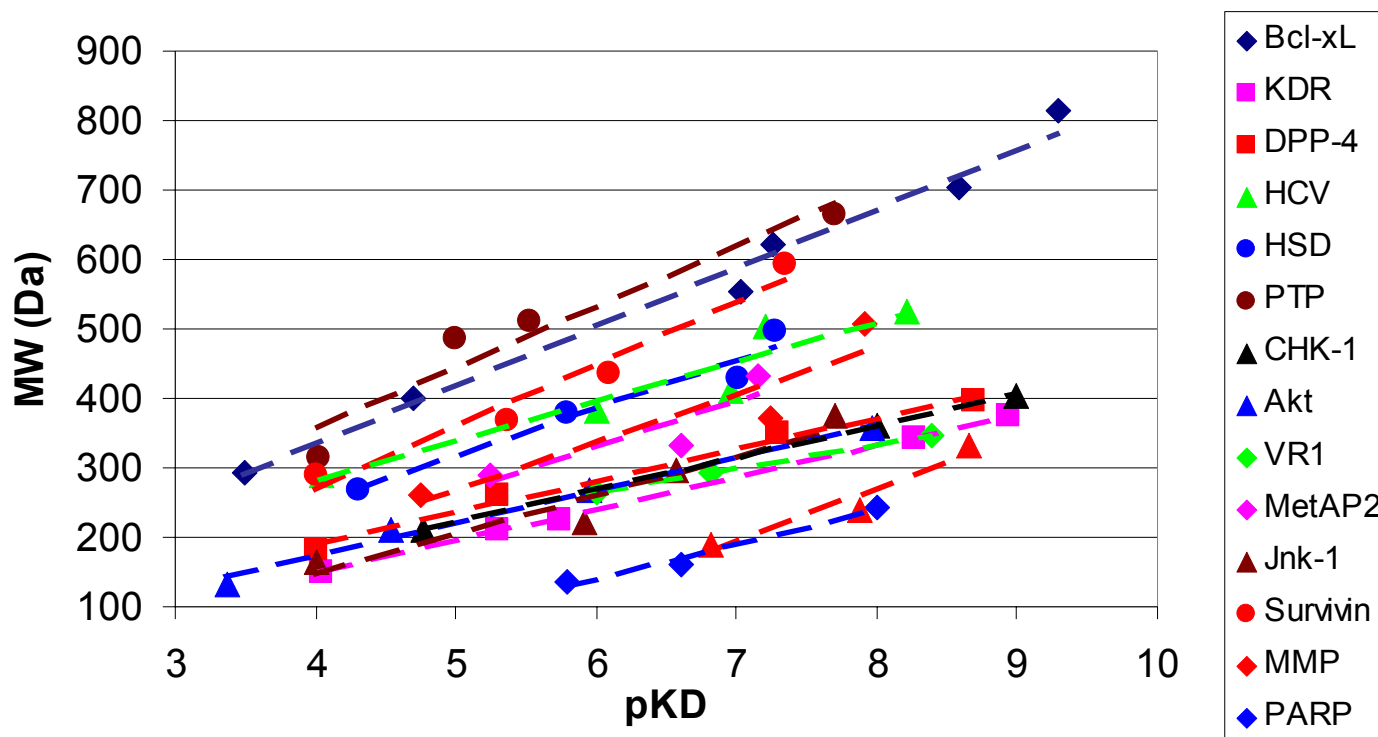
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Candidate Deconstruction – All Series

- All deconstructions are linear
- All series are co-linear
 - Average slope of 64 Da/pK_D unit

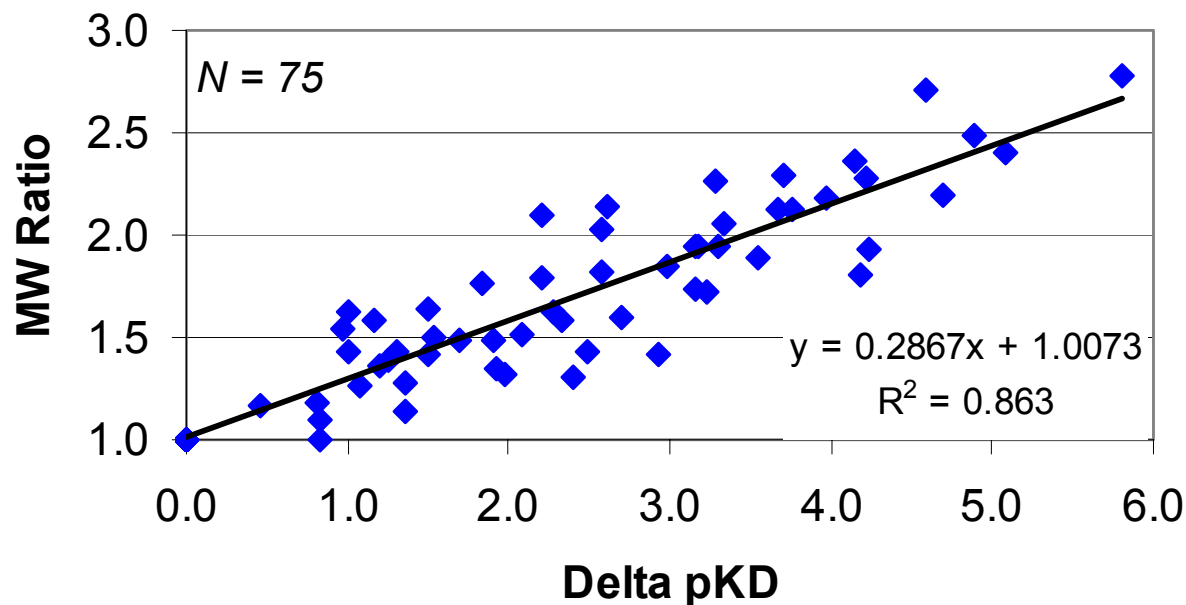


Relationship Between Mass and Potency

- If we have an optimized or nearly-optimized fragment lead, these data allow prediction of the MW of the final compound at a desired potency
- Normalize to MW of deconstructed fragment lead

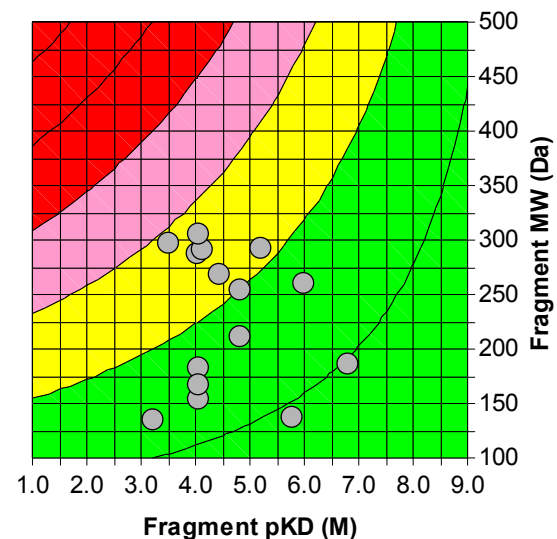
3-logs ~ 2 x MW

6-logs ~ 3 x MW



Lessons for Fragment-Based Drug Design

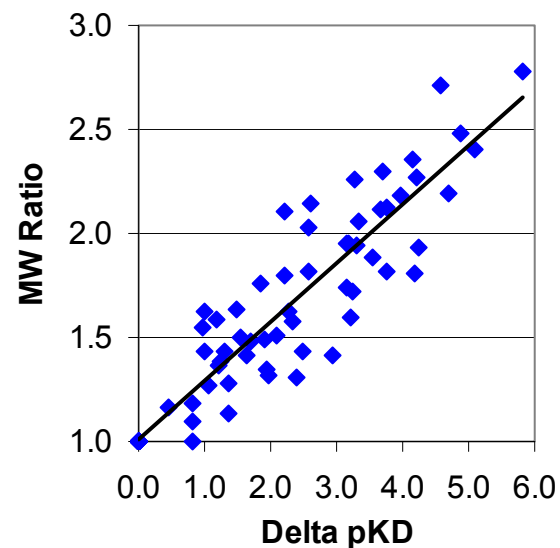
- The efficiencies of fragment leads are excellent predictors of the “druggability” of the target
 - The final mass of an inhibitor that meets defined potency criteria can be predicted with reasonable accuracy
 - Do not pursue fragment leads that fail to meet project criteria for physicochemical properties
 - Do not pursue targets that are predicted to fail project criteria for physicochemical properties
 - These data only apply to MW!!
 - Separate analysis for PSA, ClogP, etc...



Hajduk, P.J. *J. Med. Chem.* **49**, 6972-6976 (2006).

Lessons for Lead Optimization

- If you simply add mass to an invariant core:
 - Rules of thumb for expected potency for change in mass
 - ~0.3 kcal/mol per heavy atom
 - ~64 Da per log unit
 - Addition of a phenyl should get you 1.8 kcal/mol – or a factor of 20 in activity
 - So, getting a factor of 5 can be exciting, but you're probably not putting on the best substituent to fill the space



Hajduk, P.J. *J. Med. Chem.* **49**, 6972-6976 (2006).

What This Analysis Does NOT Say

- Lead optimization progresses linearly
 - No! This is painfully circuitous. But, in the final candidate, the core will form the critical interactions and every additional heavy atom will contribute positively and equally.
- There is a highly linear correlation between mass and potency
 - No! Adding the wrong mass will get you nowhere. However, the *maximum* gain per additional heavy atom will be, on average, ~0.3 kcal/mol.
- You cannot increase the binding efficiency of a lead series
 - No! In fact, you want to! But this will likely happen in the context of core modifications (i.e., heteroatom changes, ring switches, etc). This will be very difficult to do by simply adding mass.
- Identifying the appropriate lead will guarantee success
 - No! Additional interaction sites may not be accessible from any given core.

Summary and Outlook

- NMR-driven, fragment-based drug design is highly productive and can yield clinically relevant compounds even for difficult-to-drug targets
- Expanded impact through continuous improvements in both the speed and efficiency of NMR-based screening
 - Labeling methods
 - Protein consumption
 - Experimental design
- Enhancement in discovery success through an expanded understanding of molecular recognition and chemical diversity
 - File enhancement initiatives
 - Assessing protein druggability
 - Quantitative analysis of lead identification and optimization

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