Fragment-based Approaches to Inhibiting Protein-Protein Interactions: Inhibition of the Anti-apoptotic Proteins Bcl-x_L and Bcl-2

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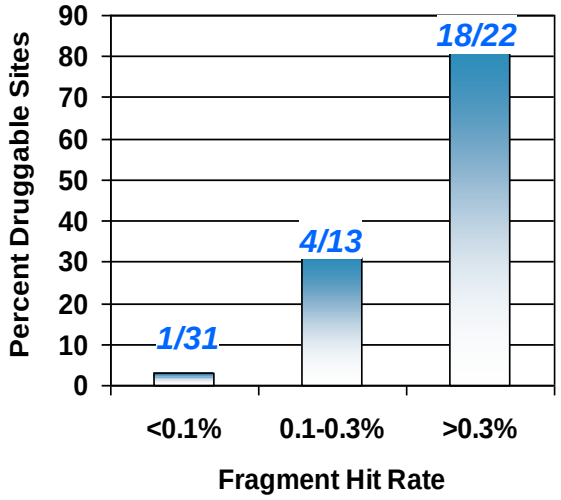
Advantages of Fragment-based Methods

- More efficient probing of chemical space with fragments
- Higher binding efficiency per atom
- Fragments find true “Hot Spot” for binding

Fragment hit rates a good measure of druggability

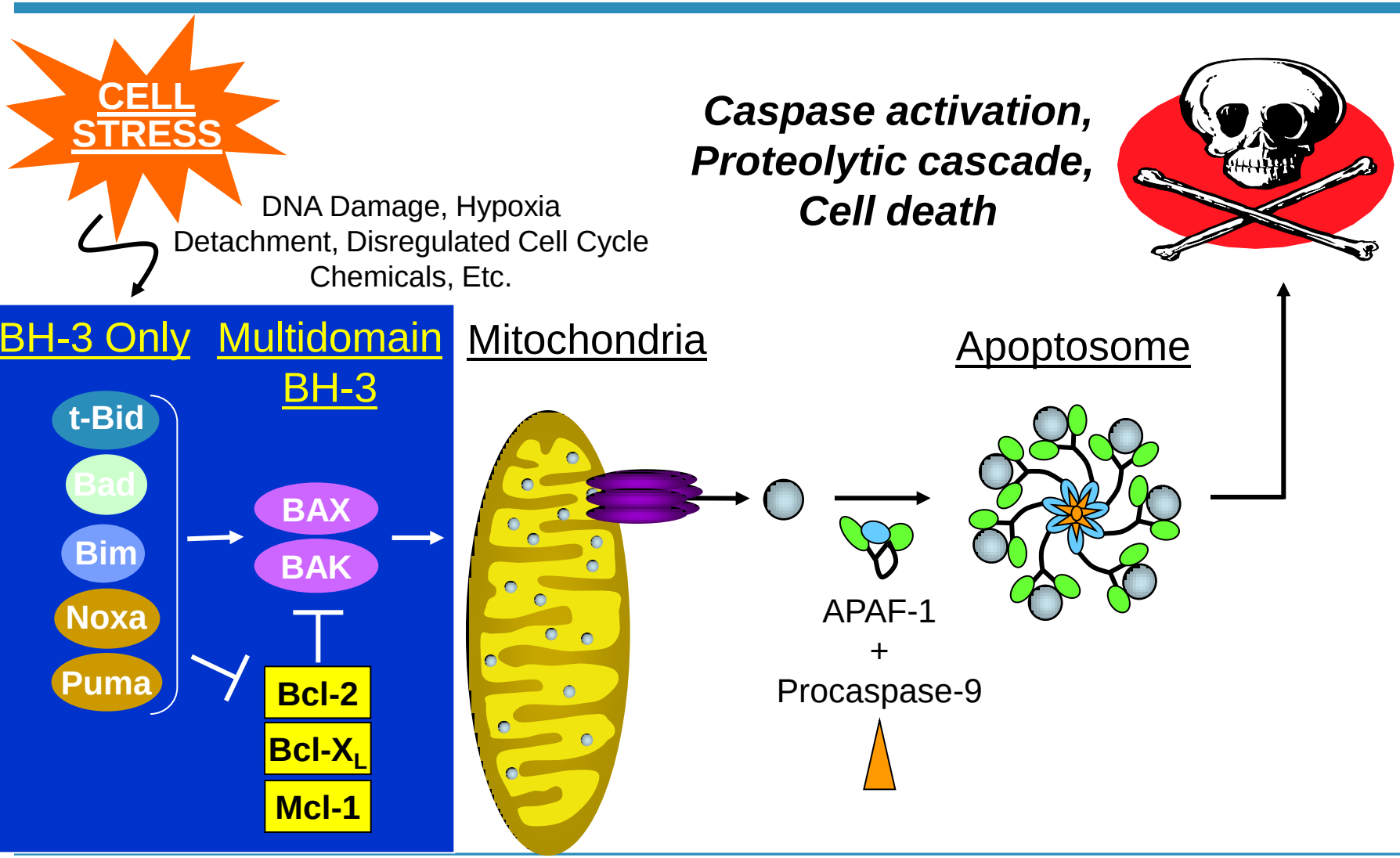
Hajduk, et al. JMC 48, 2518-2525 (2005)



Apoptosis

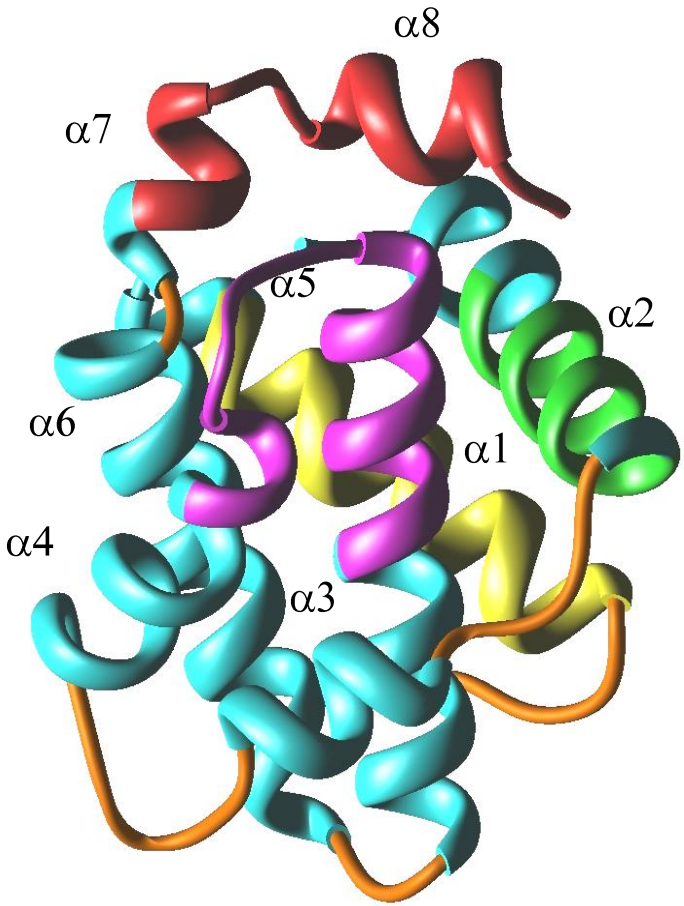
- Apoptosis, or programmed cell death, is the body's normal method of disposing of damaged, unwanted, or unneeded cells
- Important in embryonic development and for normal tissue homeosatsis
- Disruption of this process is implicated in a number of diseases including cancer

Bcl Family is the 'Gatekeeper' to Apoptotic Pathway

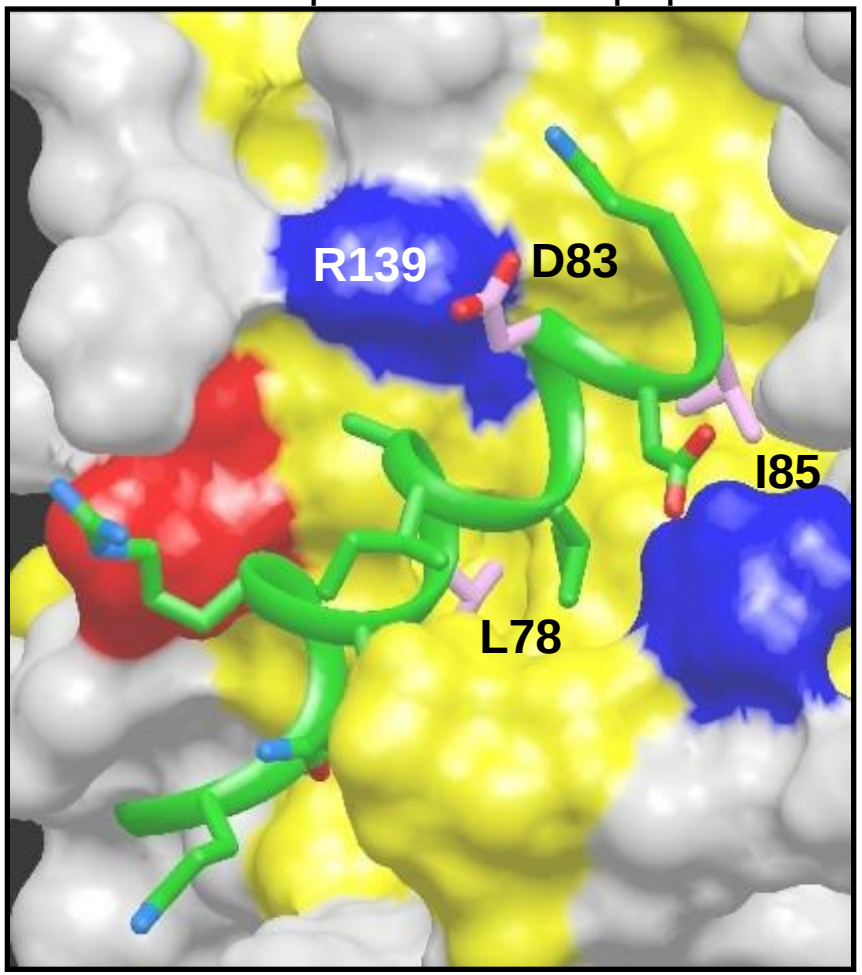


Bcl-X_L Structure

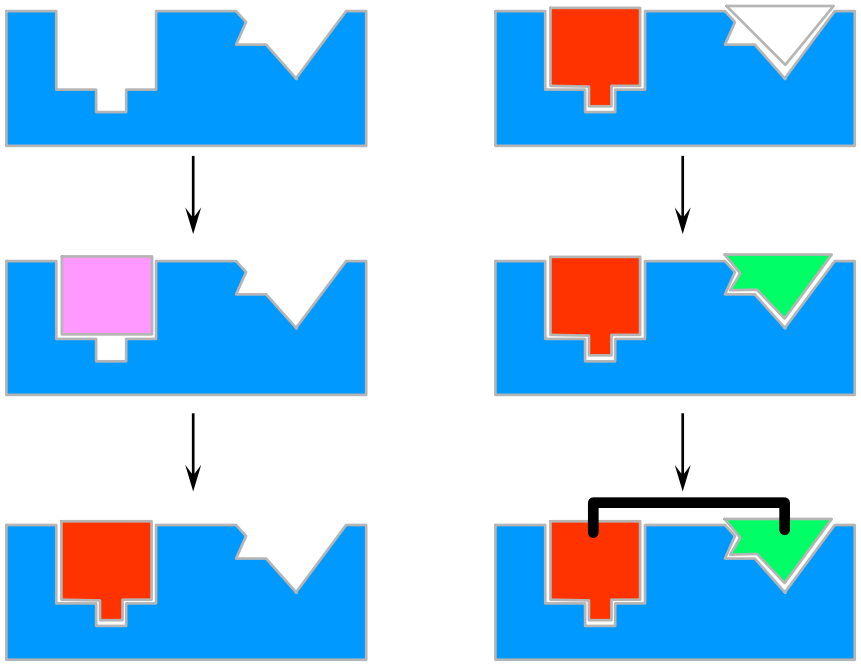
Bcl-xL



Bcl-xL complexed to Bak peptide



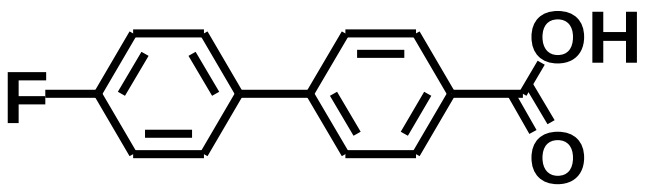
“SAR by NMR” Approach to Fragment-based Screening



$$K_D(AB) = K_D(A)*K_D(B)*L$$

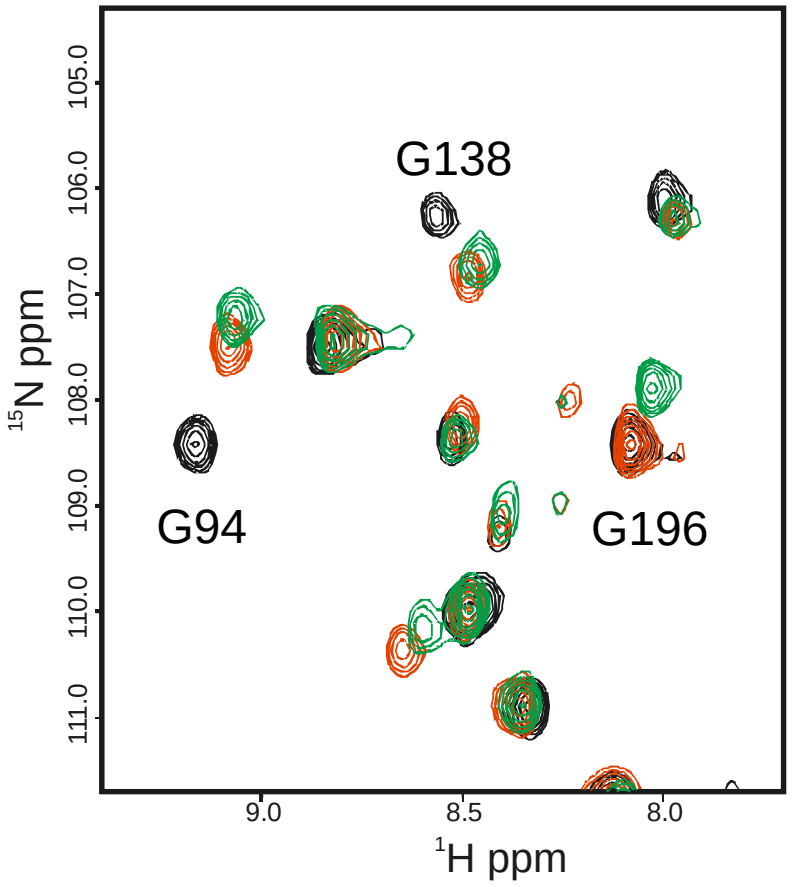
First-Site Screen

- 10,000 compound library
- <MW> ~ 215
- [Compound] = 1 mM

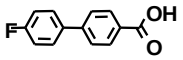
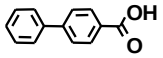
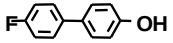
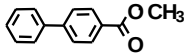
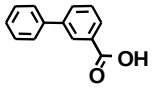
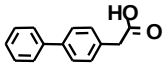
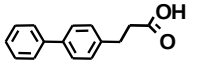
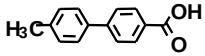
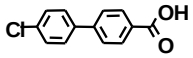
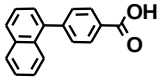


K_D = 300 μM

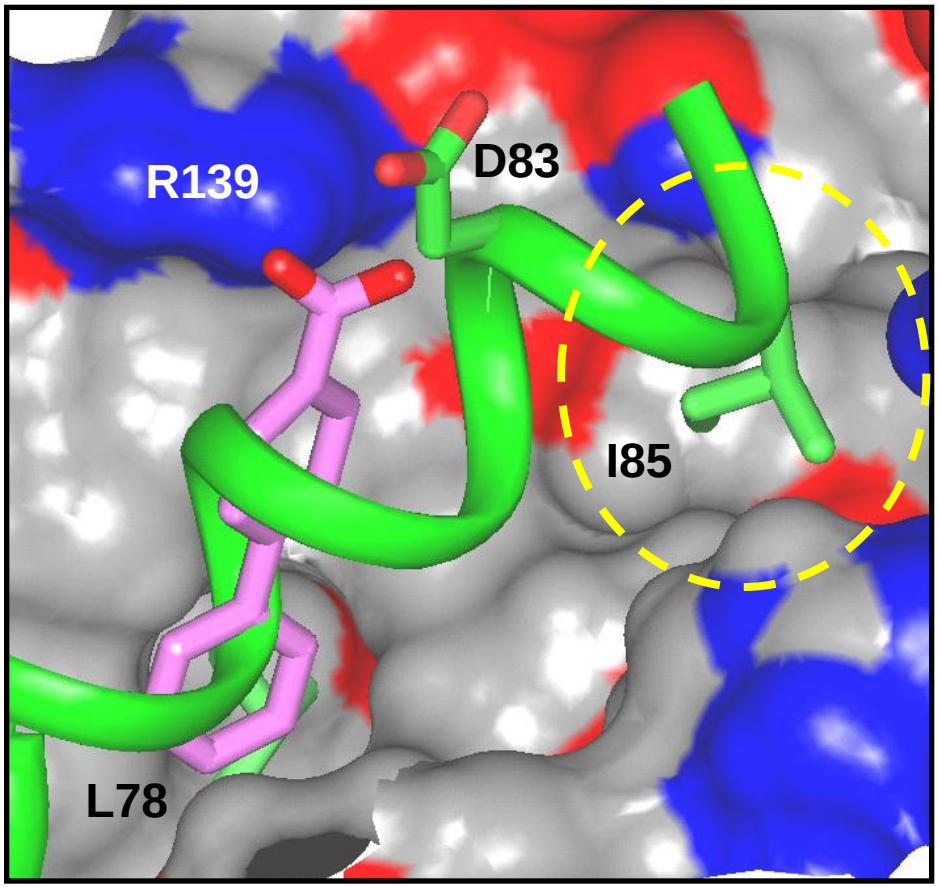
- Monitor Binding with 15N-HSQC spectrum



SAR of Biaryl Acid

No.	Structure	NMR K_d (μM)
1		300
2		1200
3		> 5000
4		> 5000
5		> 5000
6		2000
7		1990
8		383
9		238
10		250

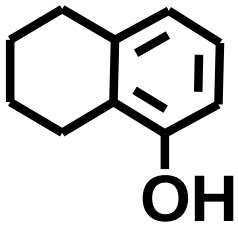
NMR Structure of Bound Fragment



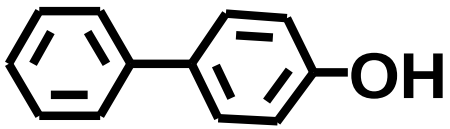
- Binds to peptide “hot spot”
  - Two key interactions maintained (Leu and Asp)
- Second site accessible
  - Ile pocket of Bak peptide

Second-Site Screen

- Screen in excess of biaryl acid
- 3,500 compound library
- <MW> ~ 150
- [Compound] = 5 mM

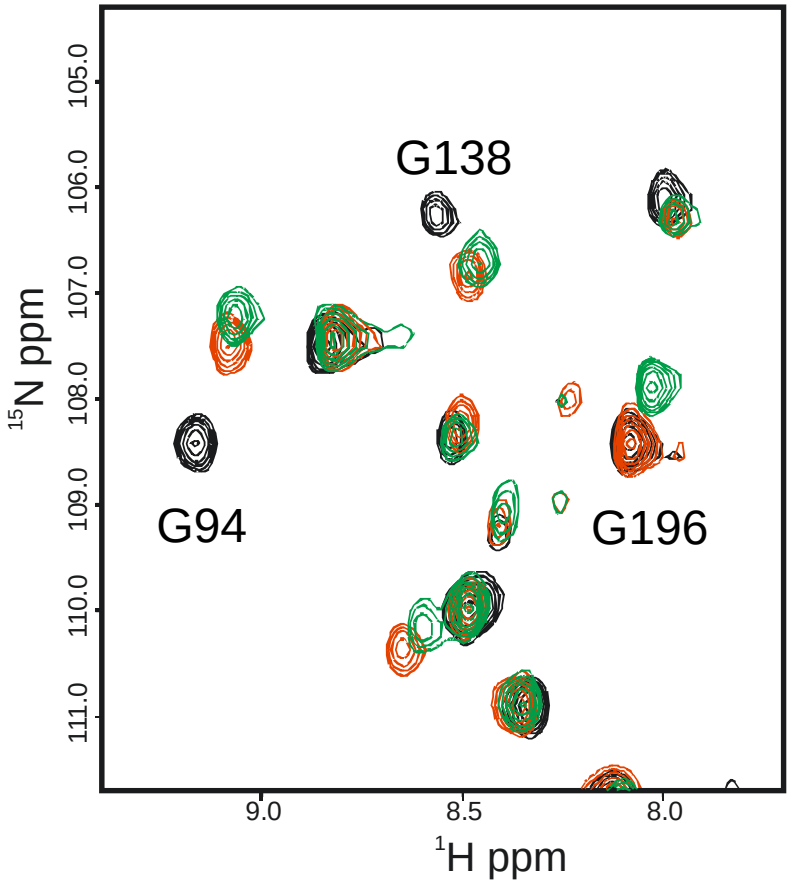


K_D = 2000 μM

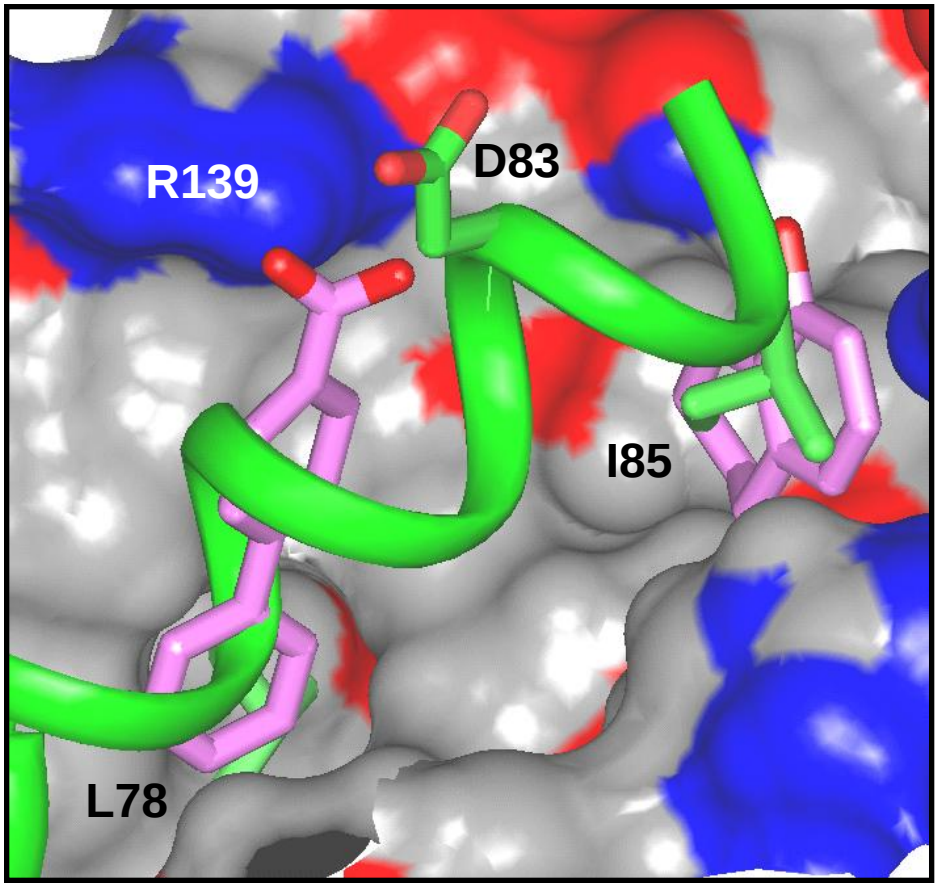


K_D = 6000 μM

- Monitor Binding with 15N-HSQC spectrum

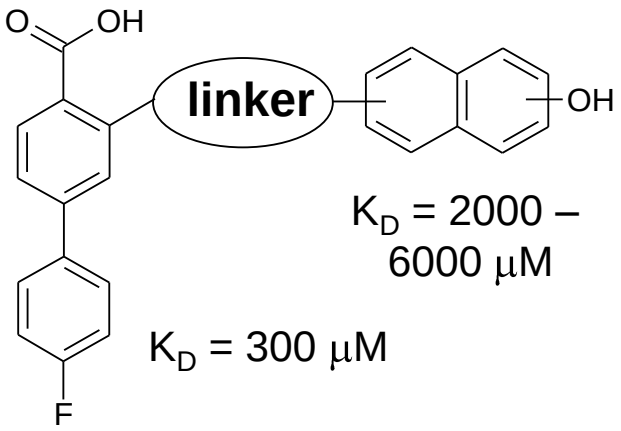


NMR Structure of Ternary Complex



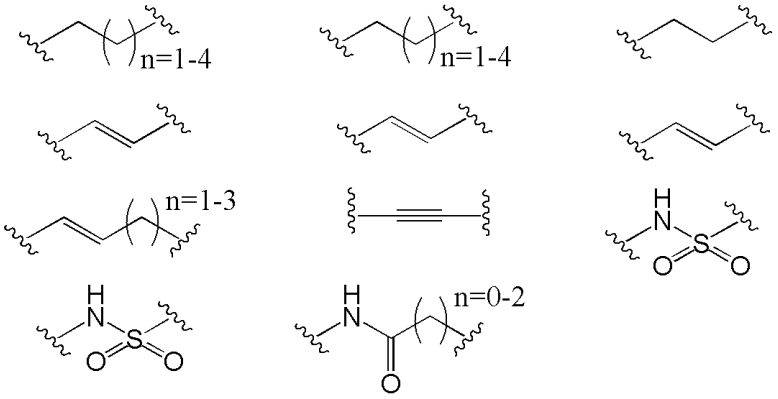
- Binds to second peptide "hot spot"
  - Ile of Bak peptide

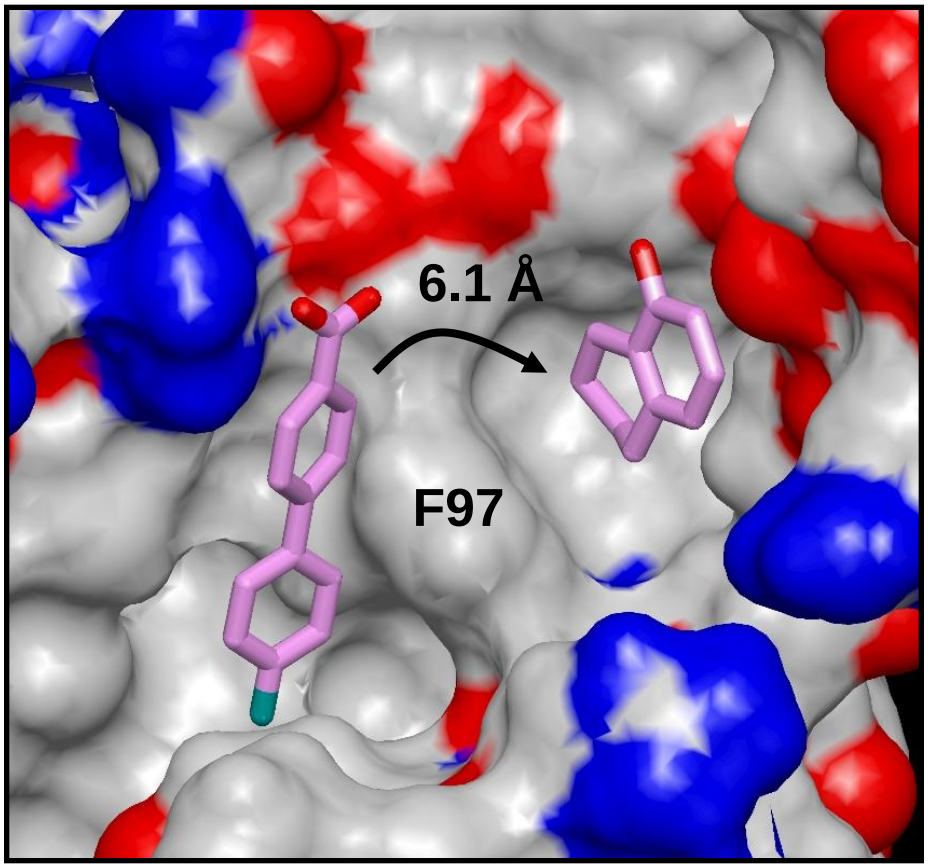
Linking Strategy



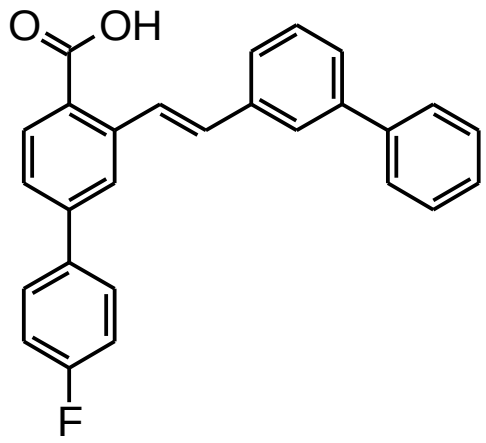
K_D = 2000 – 6000 μM

K_D = 300 μM



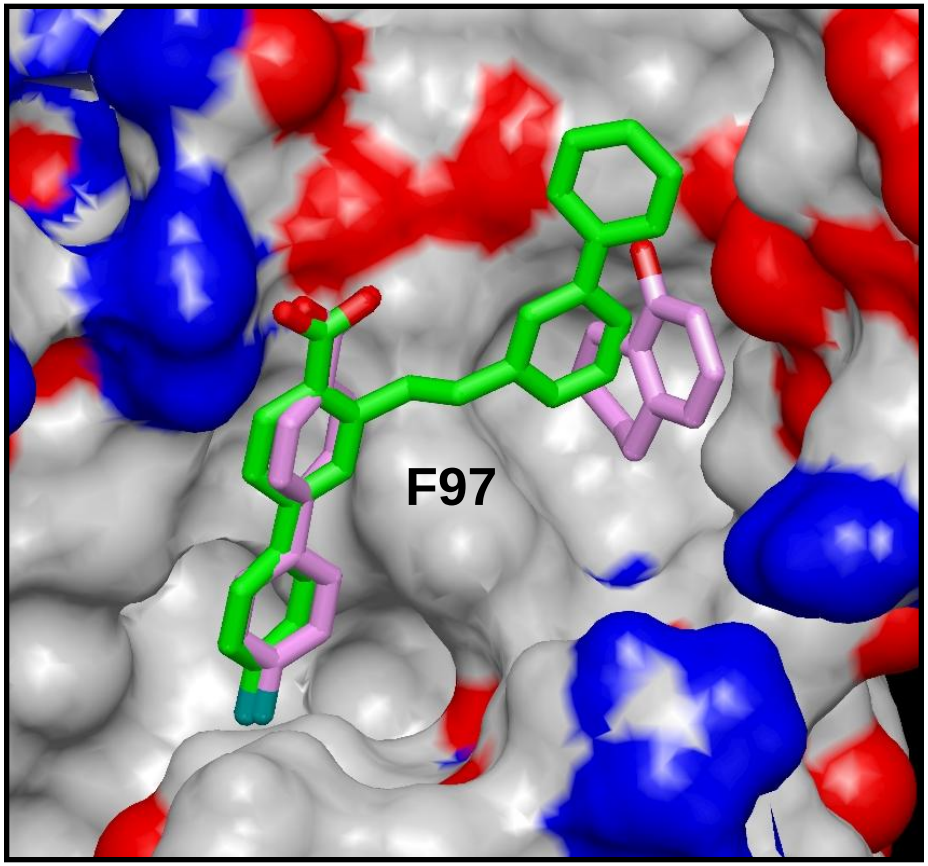


Linking Strategy

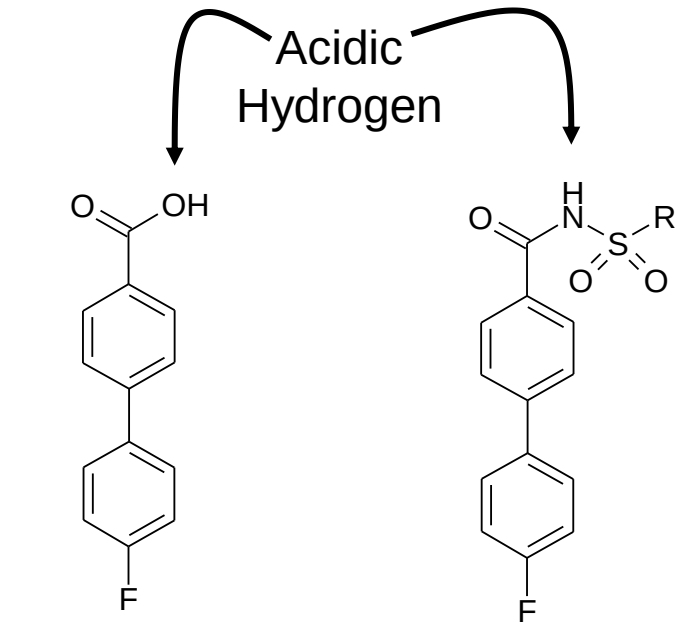


FPA IC_{50} = 1.4 µM

- Accesses hydrophobic second site
- 200-fold gain in potency
  - Expected >150-fold
- Still room for improvement



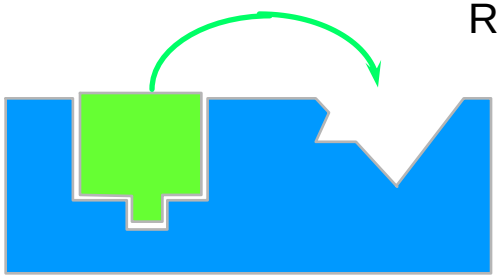
Acylsulfonamide Linking Strategy

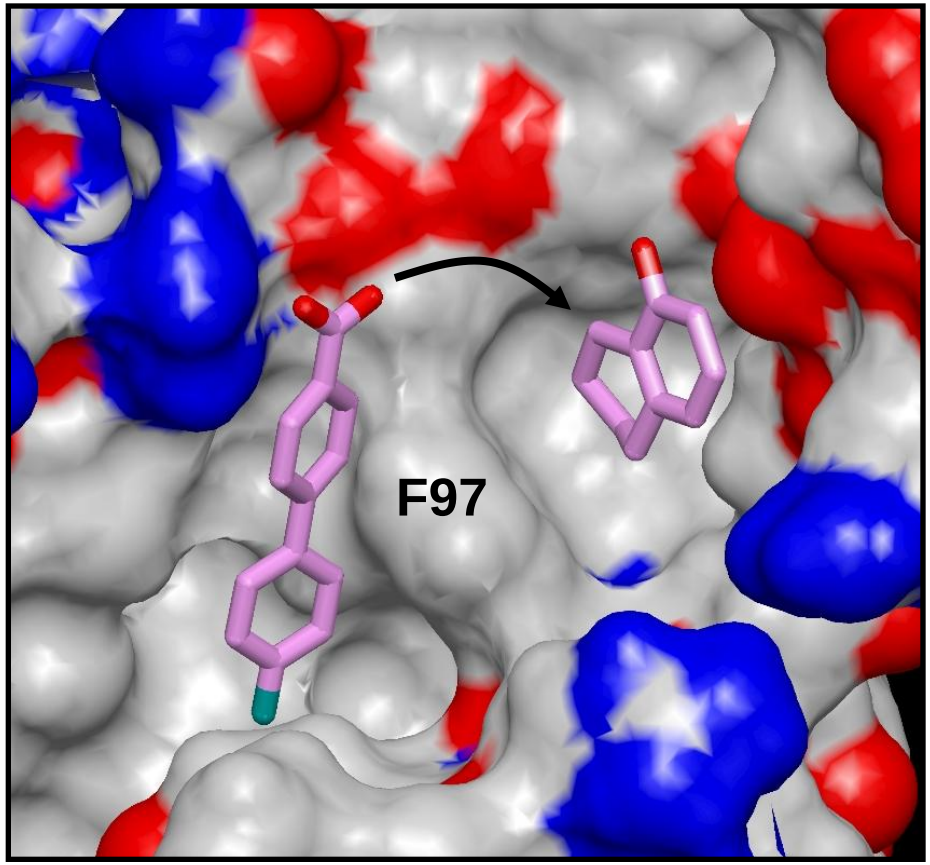


K_D = 300 µ**M**

K_D = 320 µ**M**

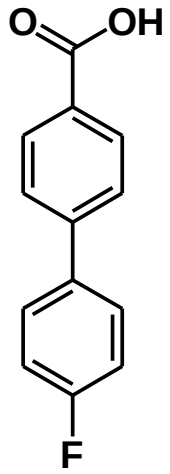
R = Me





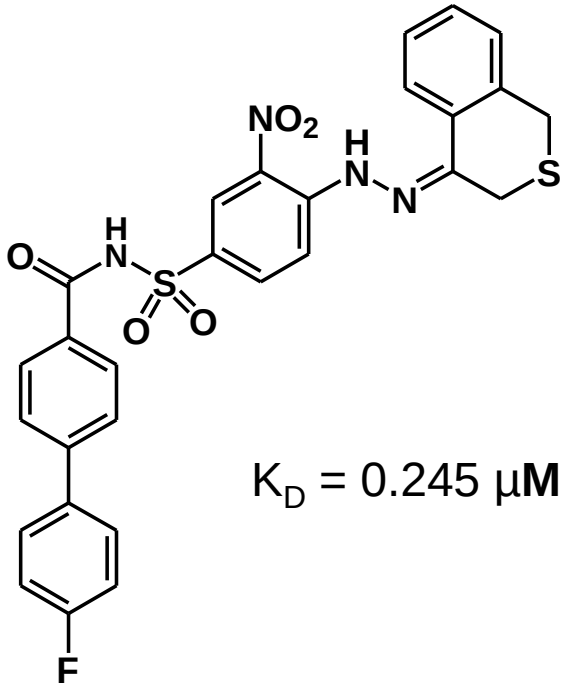
- New trajectory: avoids F97
- Maintains acidic nature

Diversity Approach to 2nd Site Binders



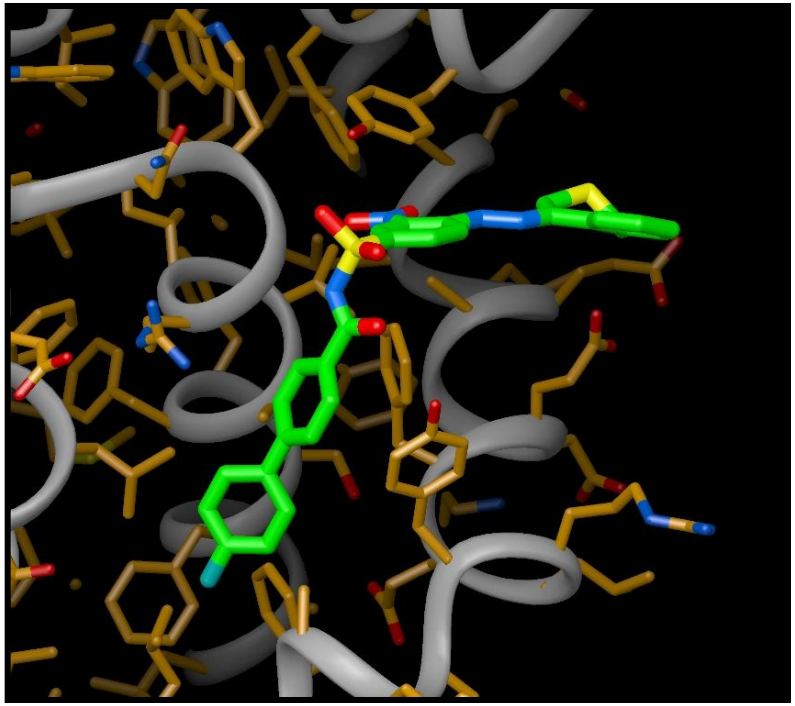
React with 120 diverse sulfonamides

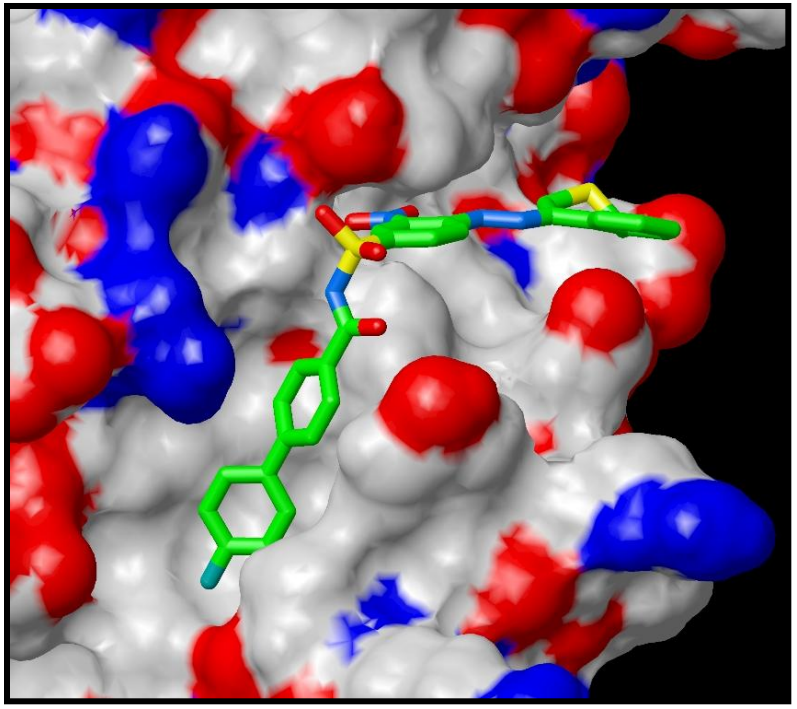
$R\text{-}SO_2NH_2$



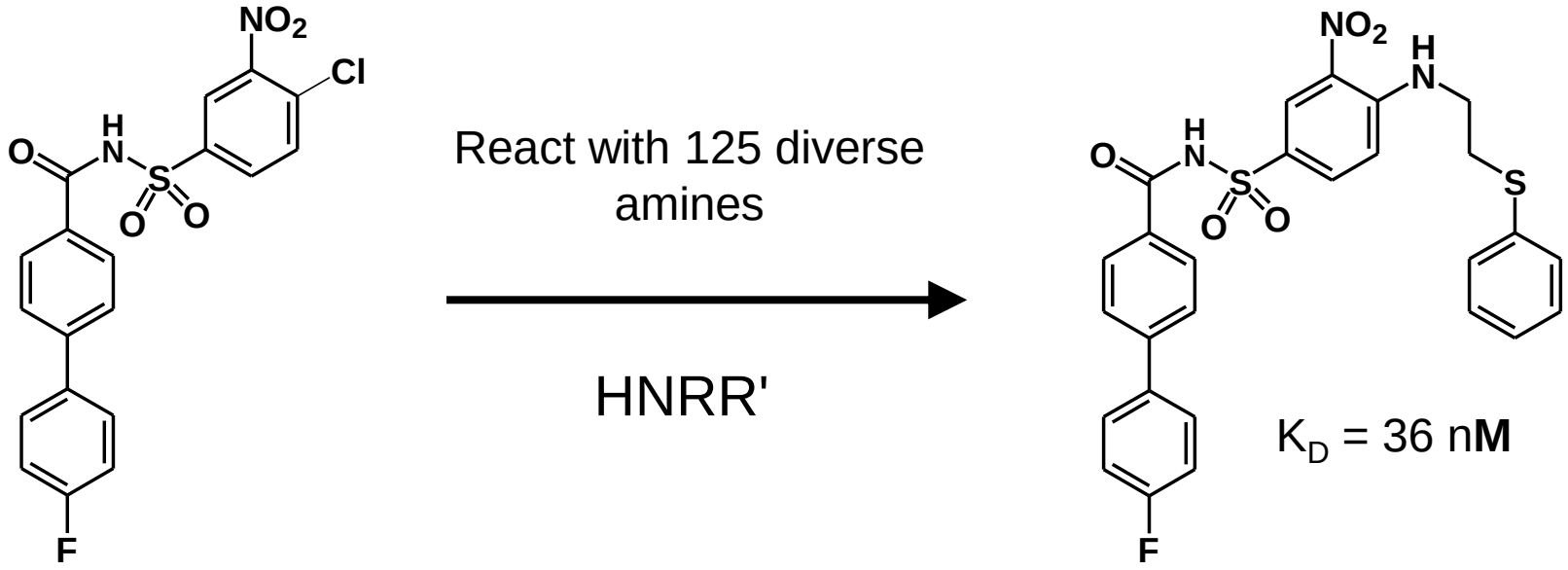
K_D = 0.245 µ**M**

NMR Structure of Acylsulfonamide

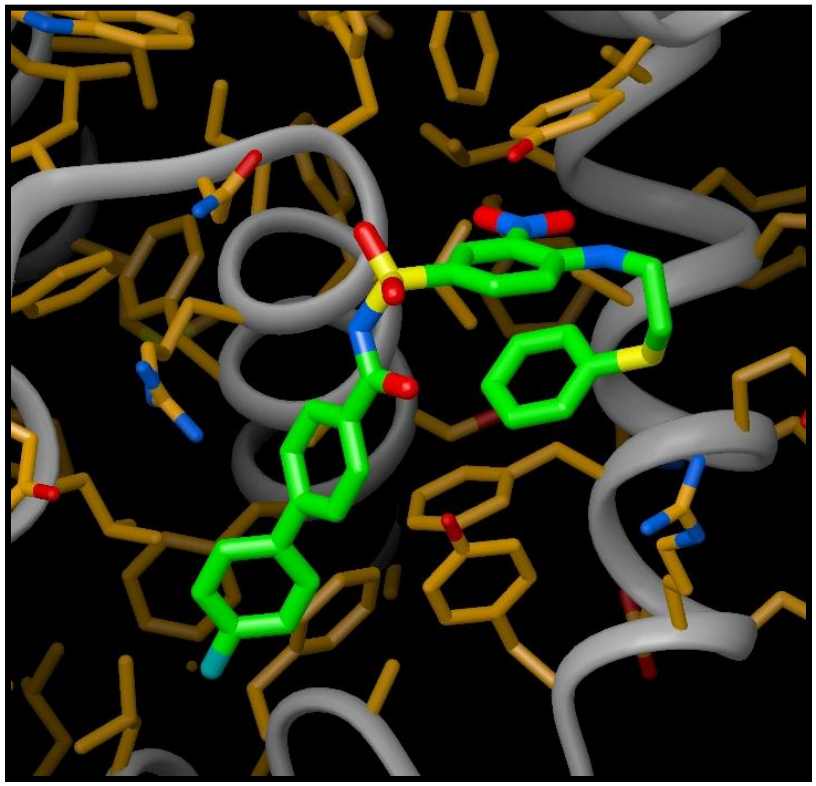


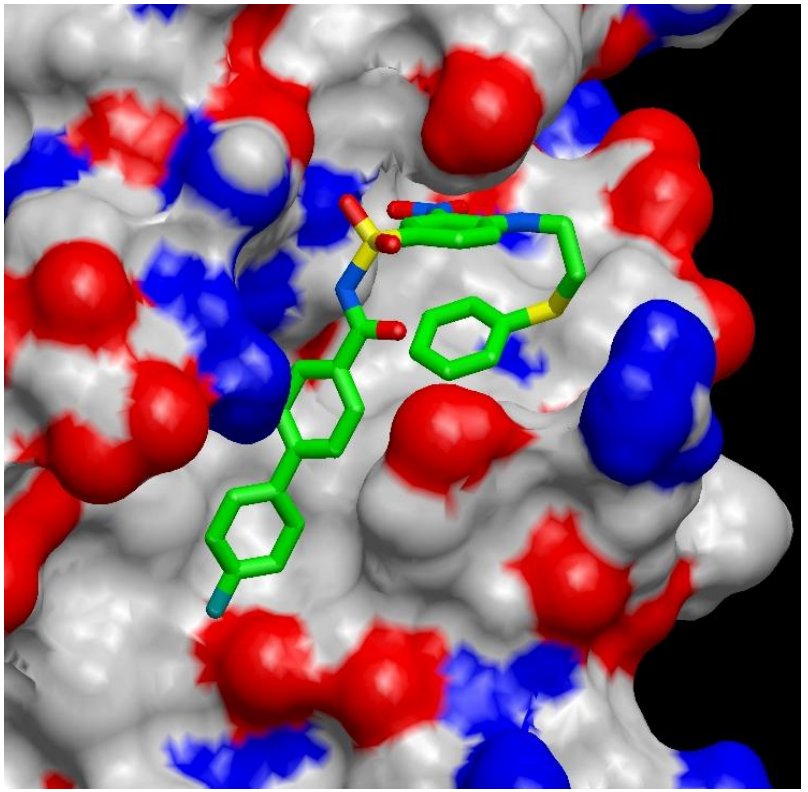


Improving Second-site Affinity

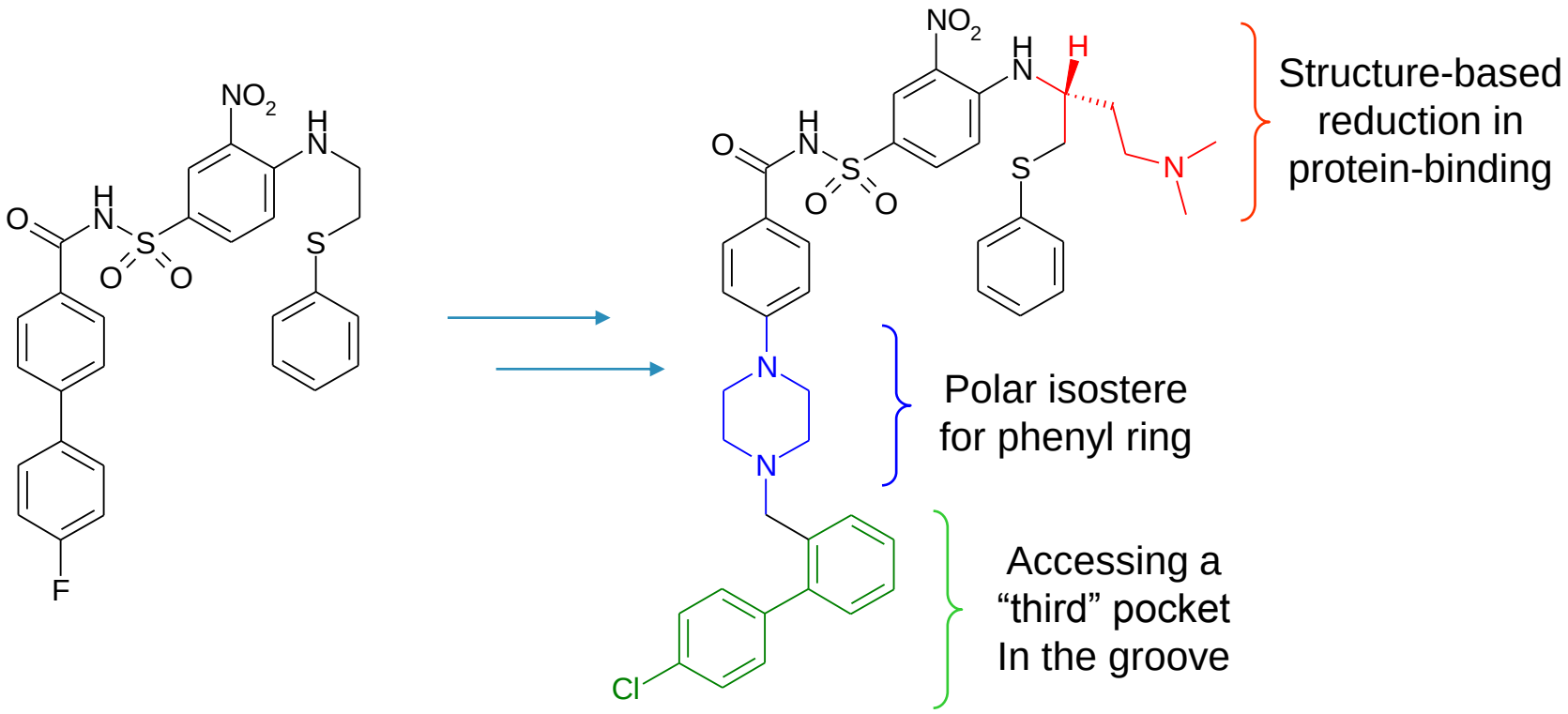


“Collapsed” Conformation Improves Ligand Affinity





Structure-Based Optimization of Bcl-x_L Inhibitors

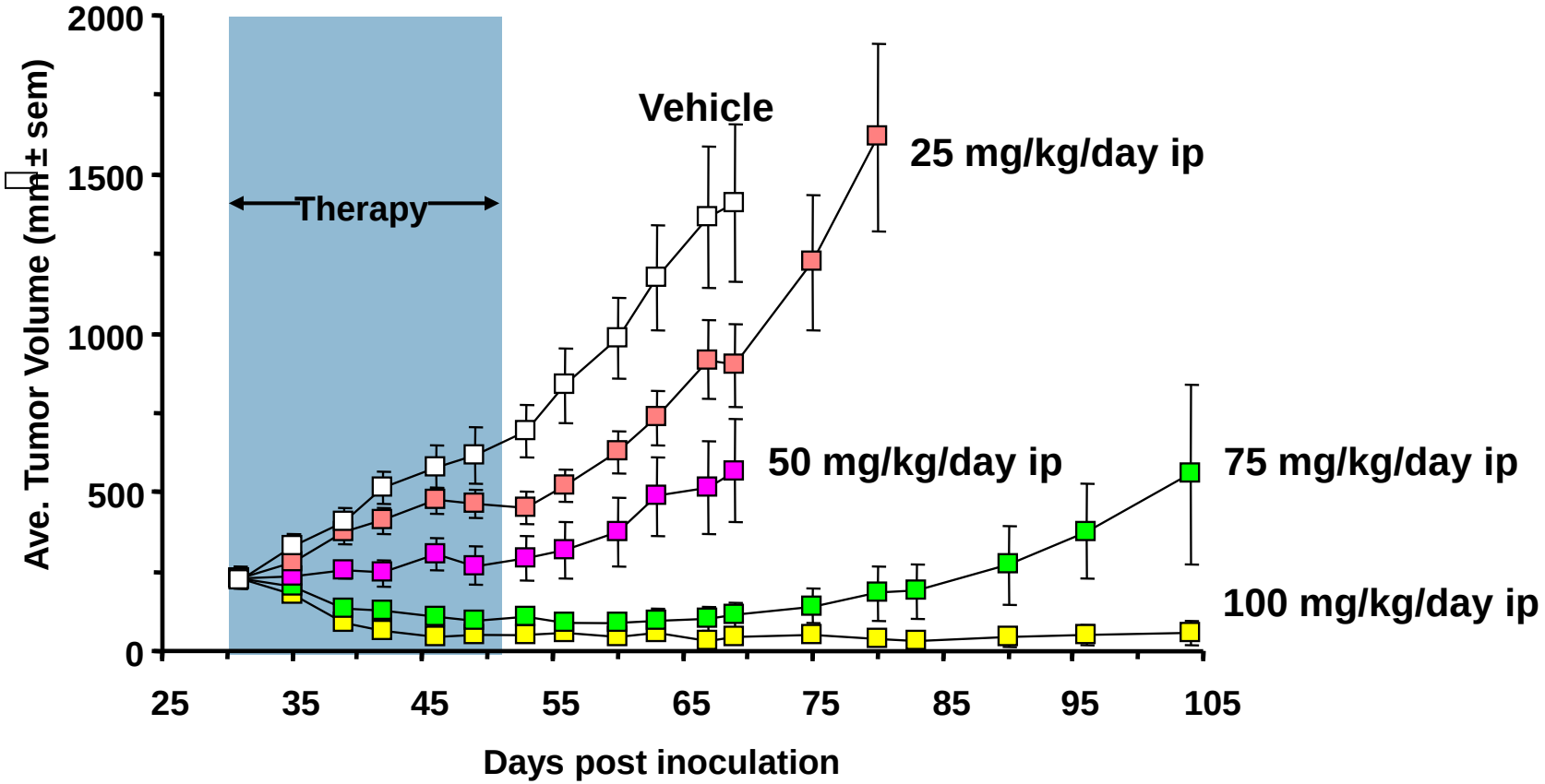


K_D = 36 nM

ABT-737
K_D < 1 nM

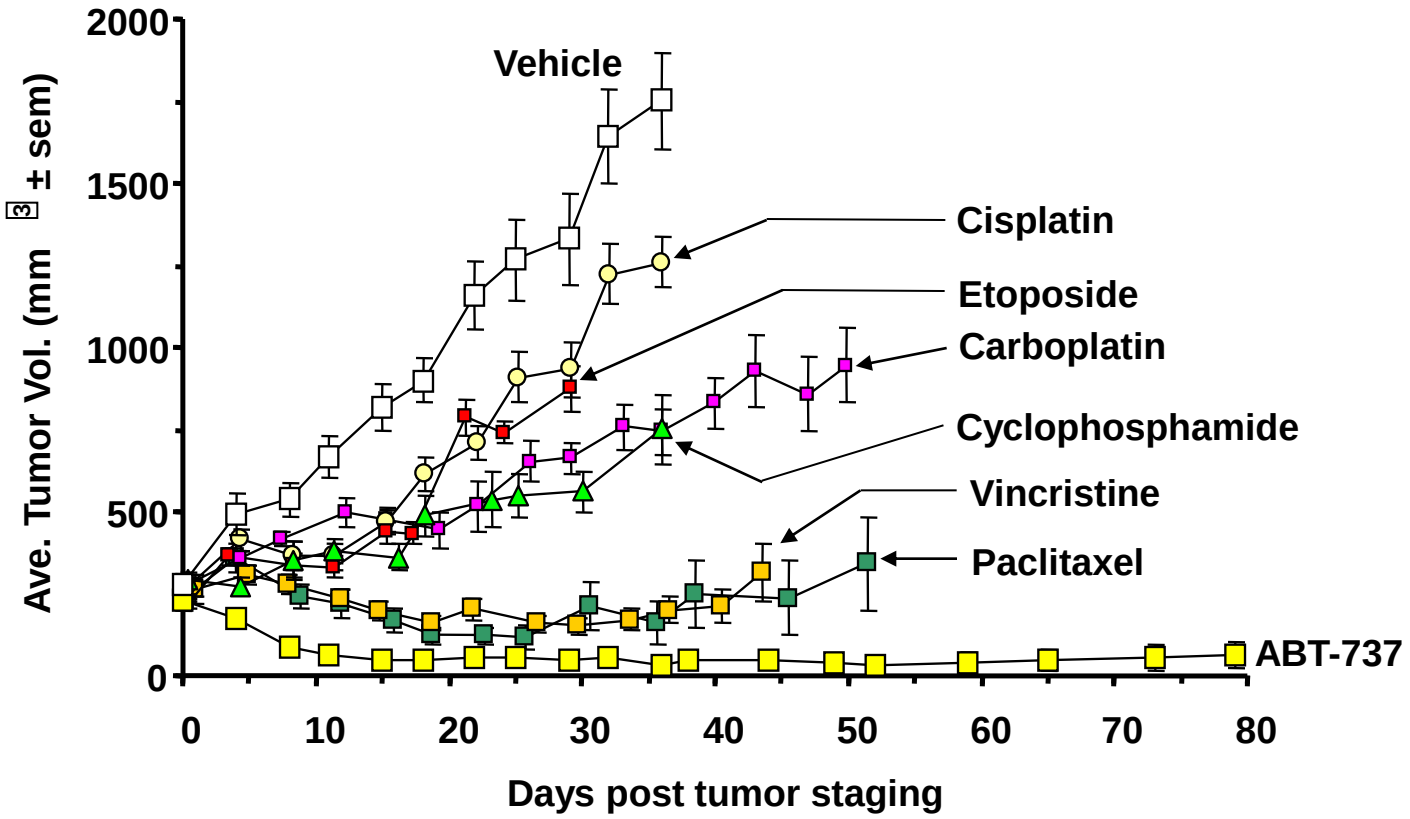
Nature **435**, 677-681 (2005)

Dose Response of ABT-737 in H146



- Treatment of established tumors (230 mm^3)
- Complete tumor regression: 7/9 (100 mg/kg) and 2/10 (75 mg/kg)
- Durable response: no tumor re-growth in any CR tumors by end of study

Activity of ABT-737 *vs.* Cytotoxic Agents in H146



- All cytotoxic agents given at (or near) their respective MTD's
- ABT-737 equivalent/superior to paclitaxel and vincristine
- ABT-737 superior to cisplatin, etoposide, carboplatin, and cyclophosphamide

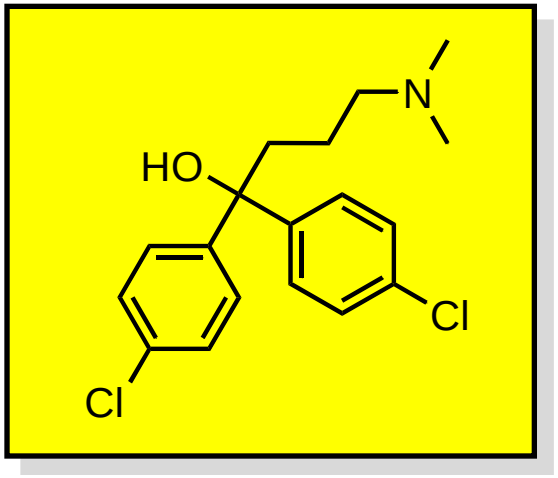
Discovery of Bcl-2 Selective Inhibitor

- ABT-737 and ABT-263 potently inhibit both Bcl-x_L and Bcl-2
- Only overt toxicity is thrombocytopenia (severe reduction in platelet count)
- This thrombocytopenia is Bcl-x_L mediated
  - Zhang et al.. Cell Death Differ 2007, 14, 943-51.
- A Bcl-2 selective inhibitor could serve as an anti-cancer agent without inducing this thrombocytopenia

First-Site Fragment Screen of Bcl-2

- Overall binding site for Bcl-2 very similar to Bcl-x_L with the exception of a few key residues

- Primary (first-site) screen of Bcl-2 led to discovery of numerous biaryl acids along with a Bcl-2 selective diphenyl methane compound

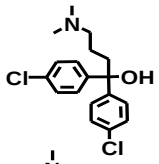
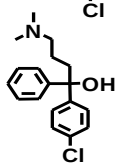
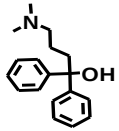
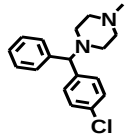
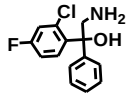


NMR K_D

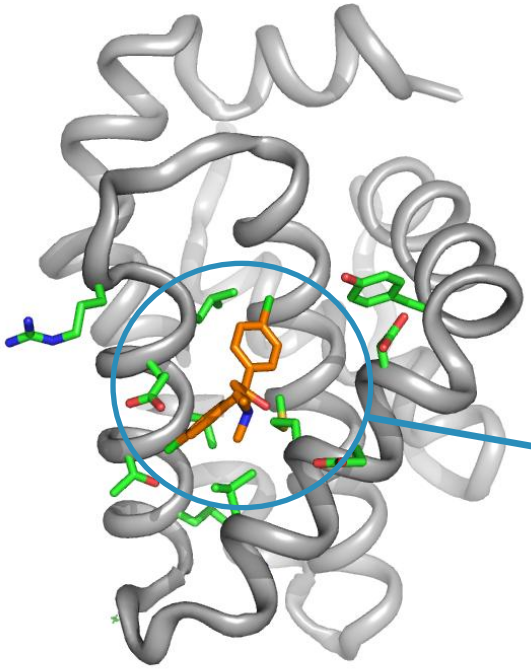
20 uM for Bcl-2

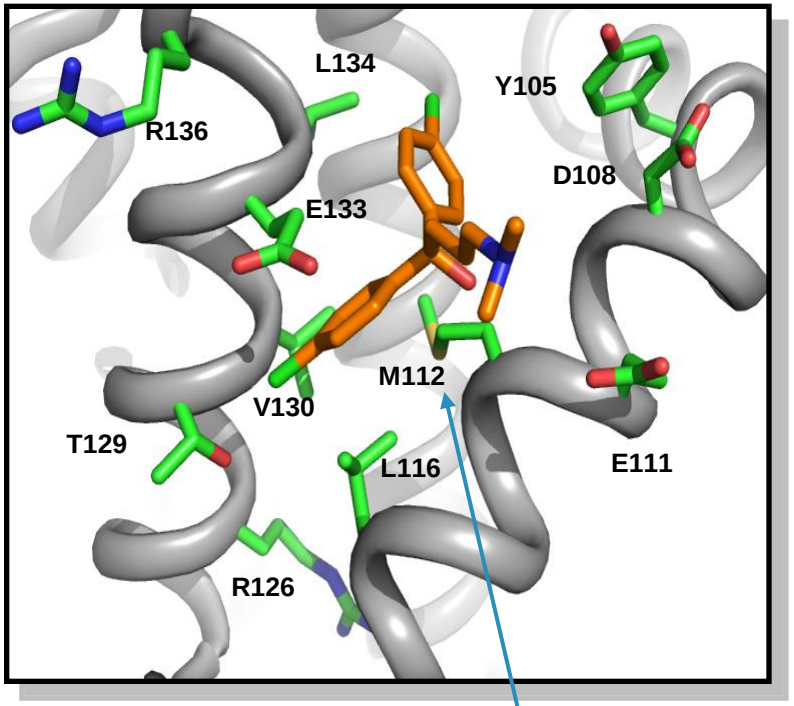
450 uM for Bcl-x_L

Diphenyl methane SAR

No.	Structure	K_D (μM) Bcl-2	K_D (μM) Bcl-x_L
1		20	450
2		80	500
3		200	5000
4		60	700
5		250	ND

NMR-derived Structure of Bound Fragment

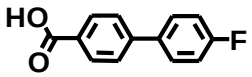
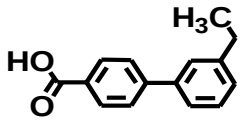
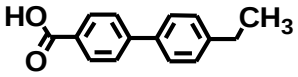




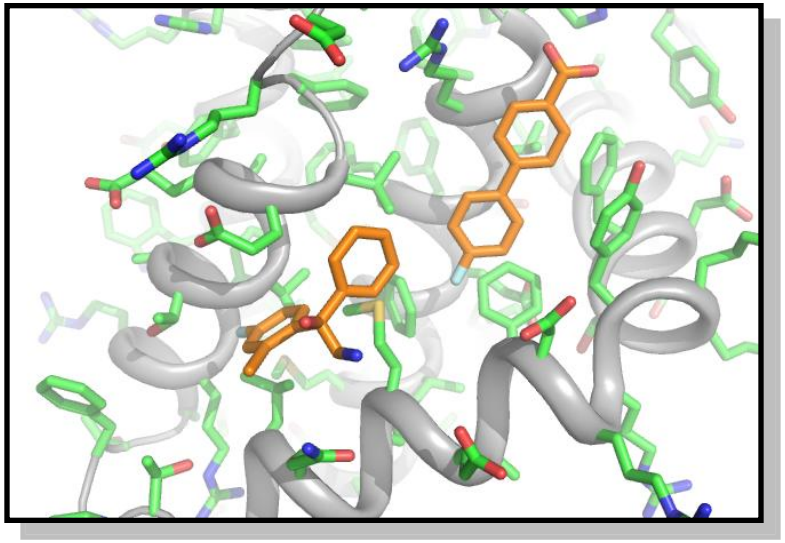
Diphenyl methane binds lower in groove than biaryl acid of Bcl-x_L

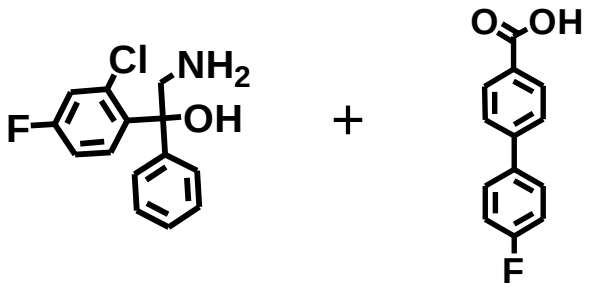
Leucine residue in Bcl-x_L

Binding of Biaryl Acids to Bcl-2

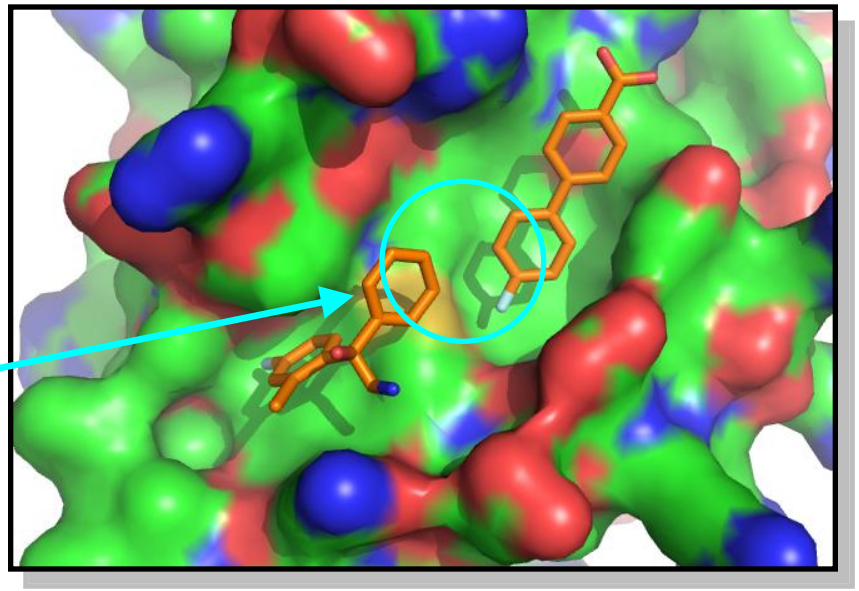
No.	Structure	Bcl-X_L K_D (μM)	Bcl-2 K_D (μM)	
			- First site	+ First site
6		300	400	430
7		290	100	20
8		360	300	> 1000

NMR-derived Structure of Ternary Complex

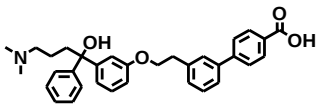
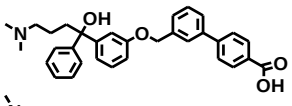
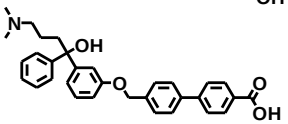
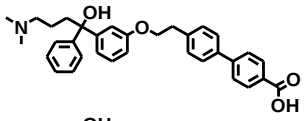
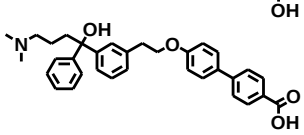


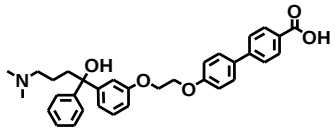
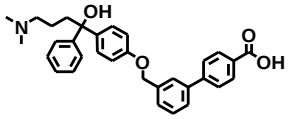
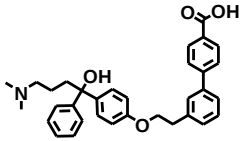
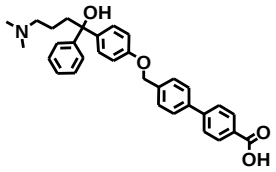
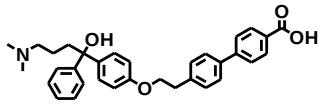
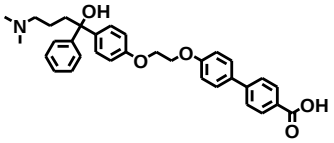


Structure of ternary complex suggests that linking could be done form either meta or para position of diphenyl methane and meta or para position of biaryl acid

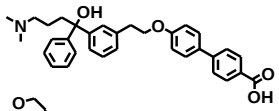
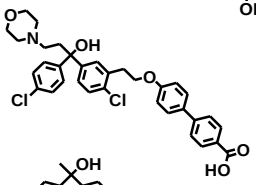
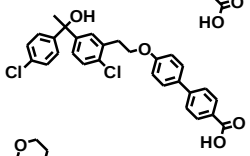
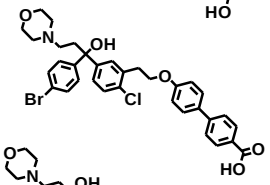
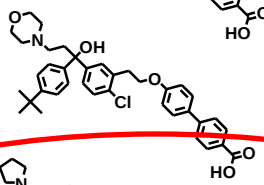
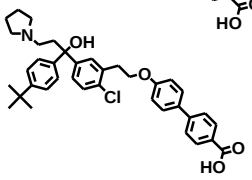


Influence of Linker Length and Geometry on Bcl-2 Inhibition

No.	Structure	K_i (μM)
13		5.0
14		20
15		5.2
16		4.6
17		3.4

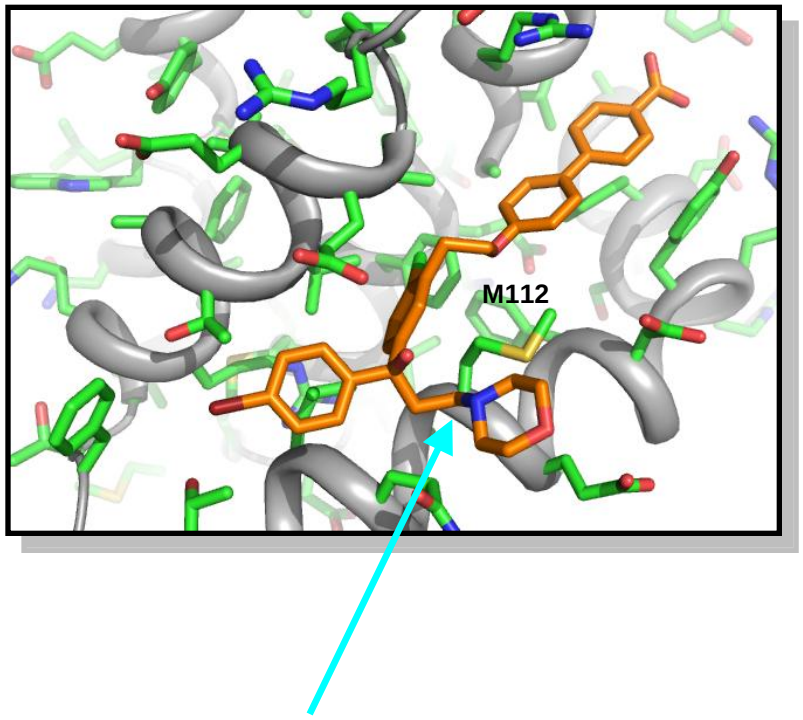
No.	Structure	K_i (μM)
18		> 1
19		5.8
20		8.0
21		83
22		12
23		> 1

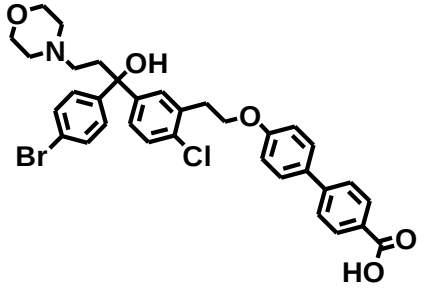
Activity of Elaborated Compounds

No.	Structure	K_i (μM)
24		3.4
25		0.22
26		0.46
27		0.20
28		0.11
29		0.04

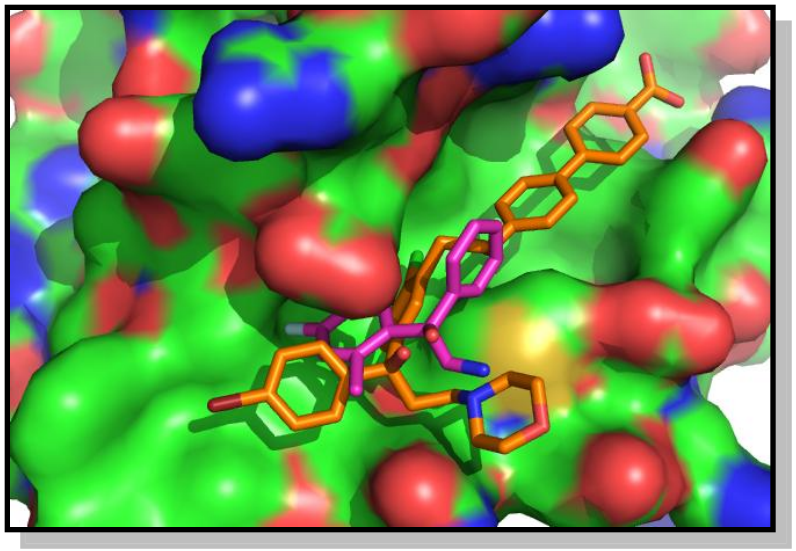
Greater than 1000-fold selectivity versus Bcl-x_L and Bcl-w, greater than 100-fold versus Bcl-B, greater than 50-fold versus A1, and about 30-fold versus Mcl-1

NMR-derived Structure of Linked Compound





Methionine flips out upon binding linked compound



Overlay with first site ligand (Magenta)

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