Targeting Protein-Protein Interactions via Target-Guided Synthesis

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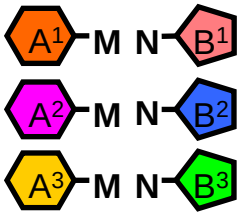
Kinetic Target-Guided Synthesis

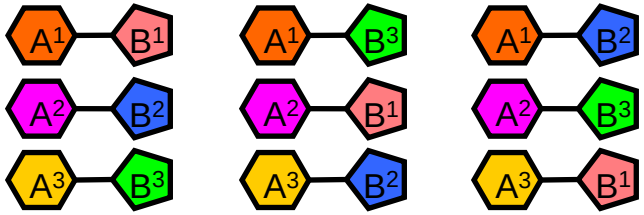
Fundamental Principle: Polyvalent interactions can be collectively much stronger than corresponding monovalent interactions.

Conventional Lead Discovery

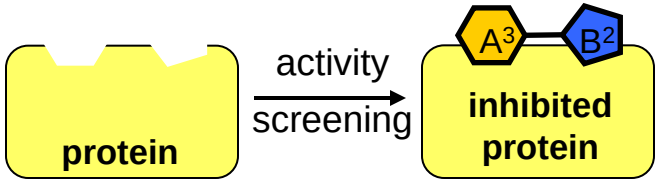
Step 1: Synthesis of building blocks



Step 2: Preparation of library of final compounds

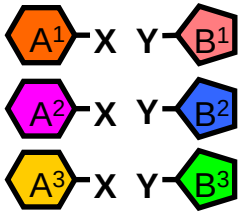


Step 3: Screening for inibition

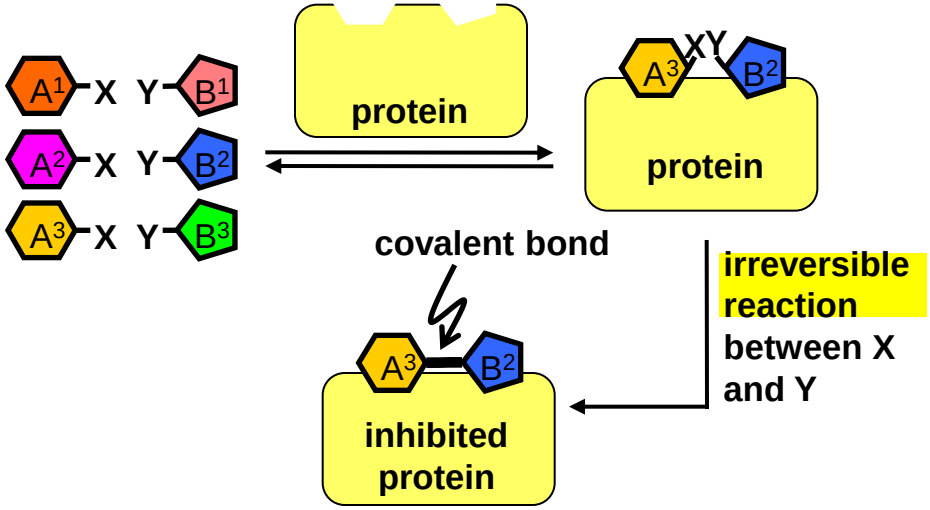


Kinetic Target-Guided Synthesis

Step 1: Synthesis of building blocks



Step 2: Screening and synthesis of biologically active compounds



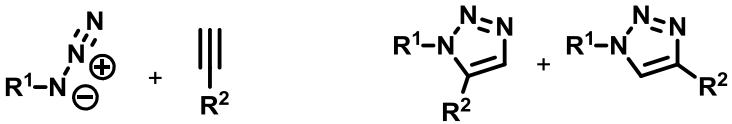
A. J. Kirby, *Adv. Phys. Org. Chem.* **1980**, *37*, 183; W. P. Jencks, *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 4046.

In Situ Click Chemistry – A Kinetic TGS Variant

Polyvalent interactions can be collectively much stronger than corresponding monovalent interactions.

Examples:
- hydrazone formation
- disulfide bond formation
- epoxide ring-opening
- N-alkylation
- S-alkylation

***In situ* Click Chemistry:**



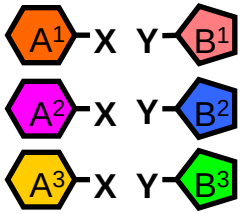
syn *anti*

- modular
- wide in scope
- high-yielding
- insensitive to oxygen and water

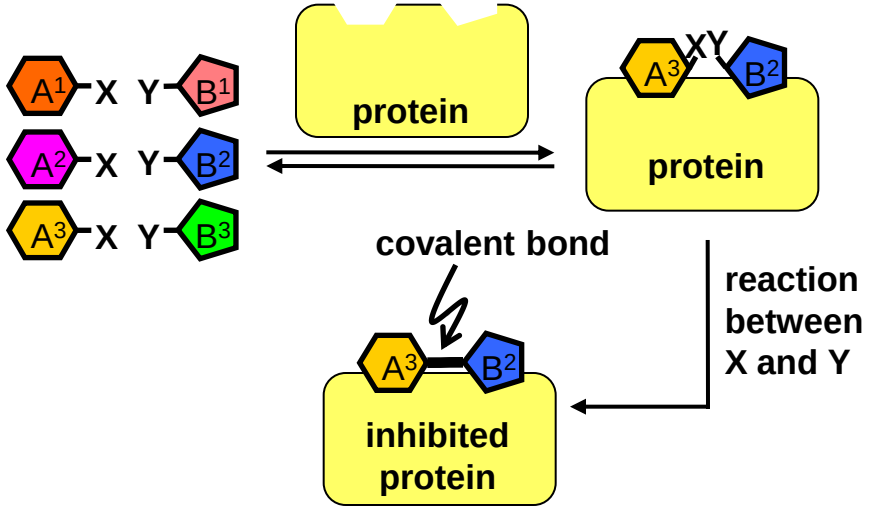
- enzymatic targets
- potent inhibitors

Target-Guided Synthesis

Step 1: Synthesis of building blocks



Step 2: Screening and synthesis of biologically active compounds



A. J. Kirby, *Adv. Phys. Org. Chem.* **1980**, *37*, 183; W. P. Jencks, *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 4046.

In Situ Click Chemistry: Kinetic TGS on Enzymatic Targets

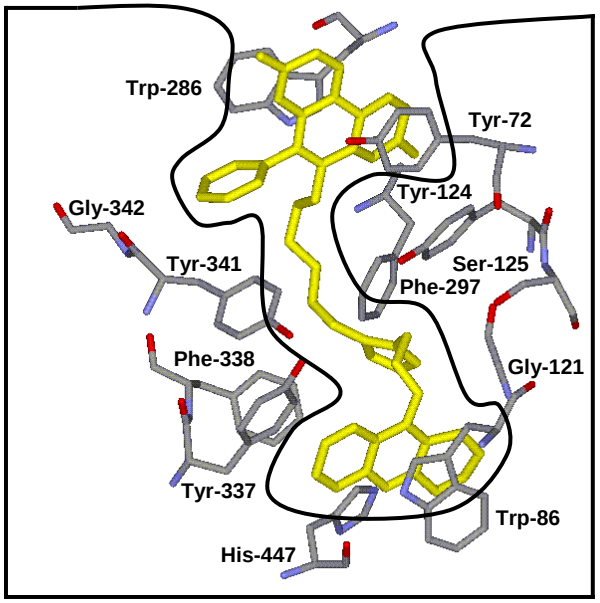
- good hit rate – known inhibitors as reactive building blocks
- very potent hit compounds
- reactive building blocks are binding with good/high affinity

for mouse AChE

TZ2 K_d = 23 nM

PA6 K_d = 360 nM

***syn*-TZ2PA6** K_d = 410 fM



In Situ Click Chemistry: Kinetic TGS on Enzymatic Targets

- good hit rate – known inhibitors as reactive building blocks
- very potent hit compounds
- reactive building blocks are binding with good/high affinity

Questions

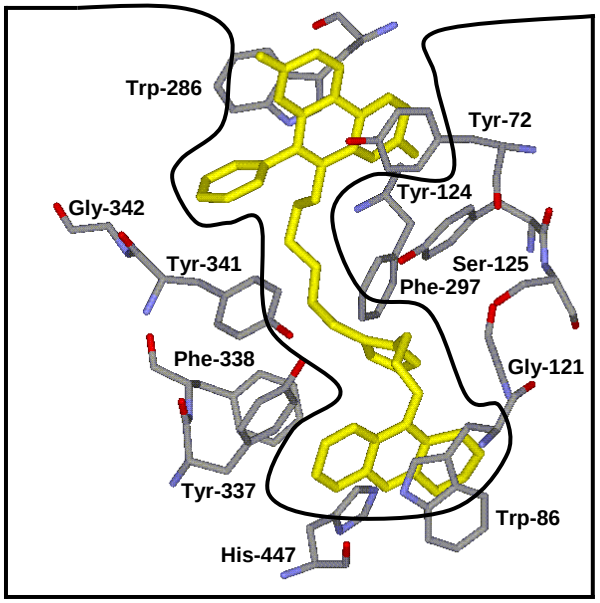
- Is kinetic TGS suitable for fragment linking?
- Is kinetic TGS suitable for challenging targets? Protein-protein interactions?
- Has kinetic TGS potential to be a straightforward fragment-based approach for targets limited in structural information?

for mouse AChE

TZ2 K_d = 23 nM

PA6 K_d = 360 nM

***syn*-TZ2PA6** K_d = 410 fM



Disrupting Protein-Protein Interactions

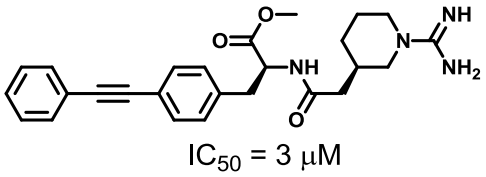
Theory of hot spots:

Subregions of protein-protein interfaces that contribute significantly to the overall free energy of binding between the proteins

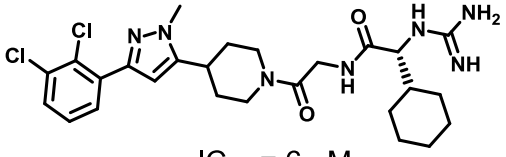
Size of hot spots is comparable to the surface area of drug-like molecules

Flexible amino acid residues at the protein surface

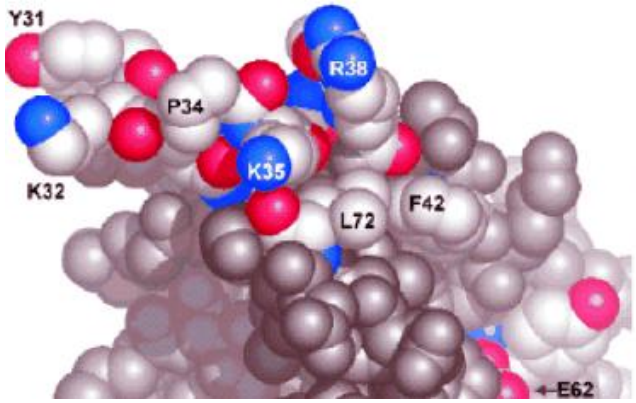
Example: Interleukin-2 (IL-2) and its receptor IL-2αR
Compounds bind to the IL-2αR-binding region on IL-2

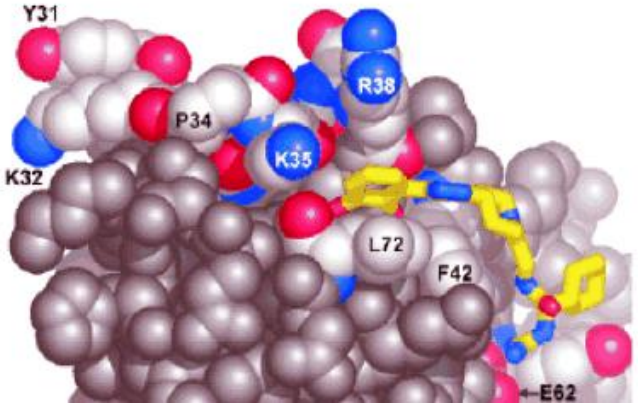


IC_{50} = 3 μM



IC_{50} = 6 μM





T. Clackson, J. A. Wells, *J. Am. Chem. Soc.* **1995**, *267*, 383.

The Bcl-2 Family: Apoptotic Switches in Cancer

Bcl-2 Family Proteins

- anti-apoptotic Bcl-2 family proteins: Bcl-2, Bcl-X_L and Mcl-1
- pro-apoptotic proteins: Bax, Bak and BH3-only proteins (Bim, Bad, Bid, Bik)

Category 1: anti-apoptotic

Bcl-2, Bcl-X_L Bcl-w, Mcl-1, A1

Category 2: multidomain pro-apoptotic

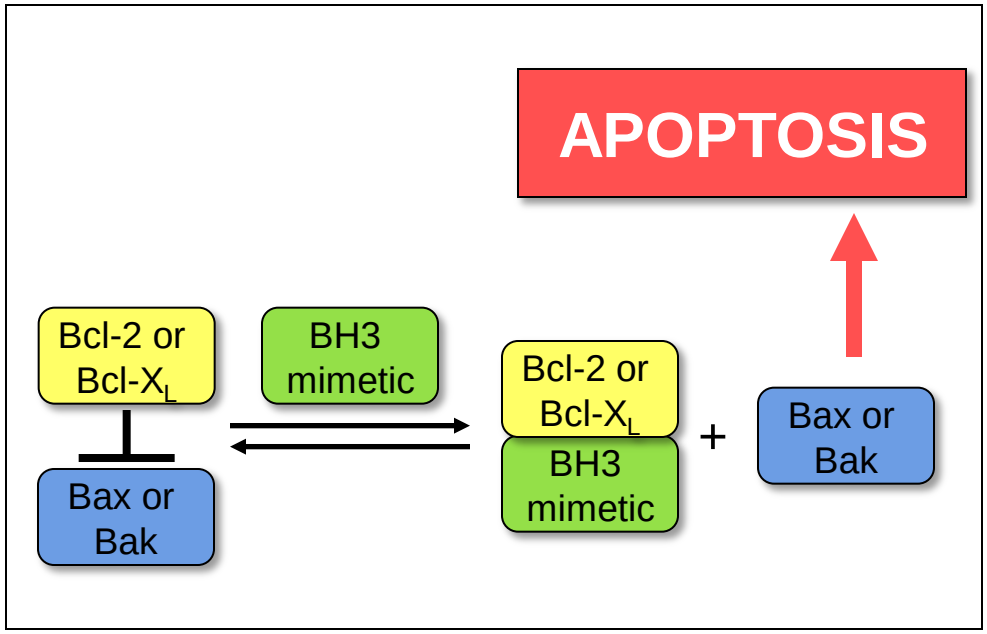
Bax, Bak

Category 3: BH3-only pro-apoptotic

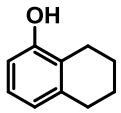
Bad, Bid, Bim, Noxa, Puma

Bcl-2 Cancer Biology

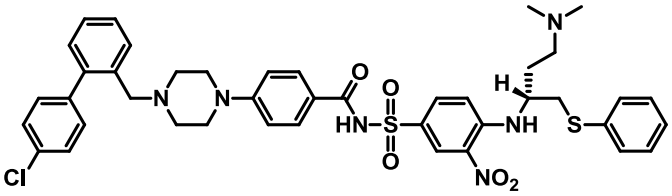
- central regulators of apoptosis (validated targets)
- contribute to tumor initiation, progression and resistance to therapy
- disrupting heterodimerization of anti- and pro-apoptotic proteins inhibits anti-apoptotic function of Bcl-2, Bcl-X_L, and Mcl-1



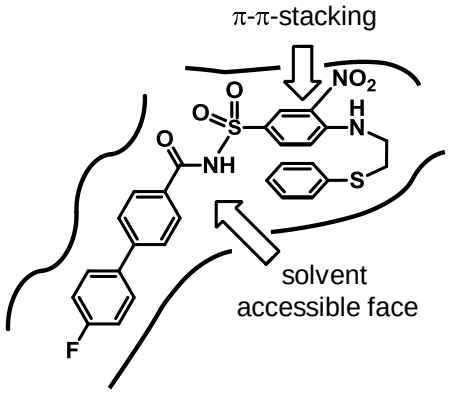
ABT-737 and Analogues



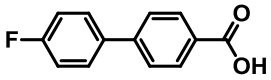
K_D = 4.3 mM



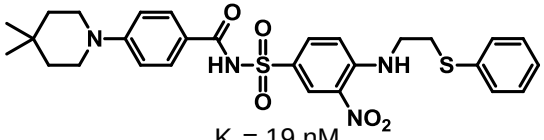
ABT-737: K_i < 2.0 nM



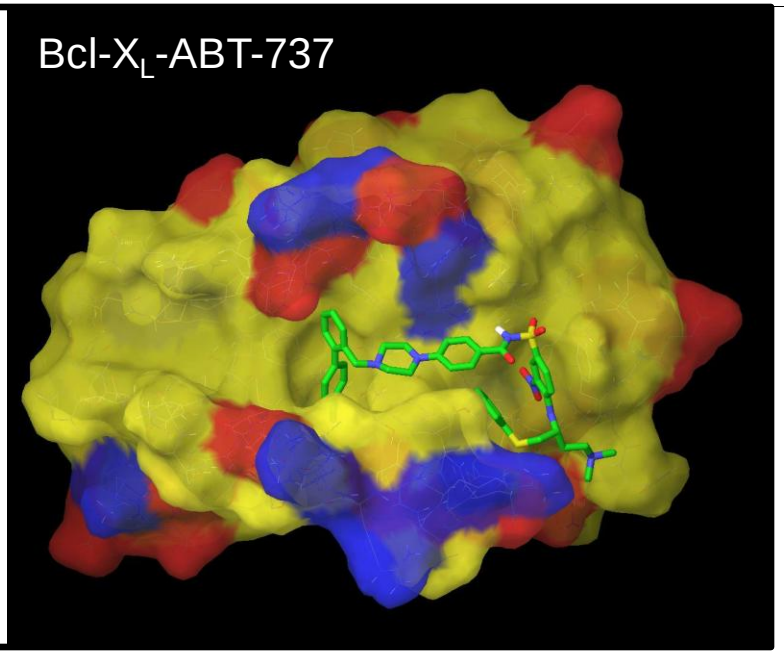
K_i = 36 nM



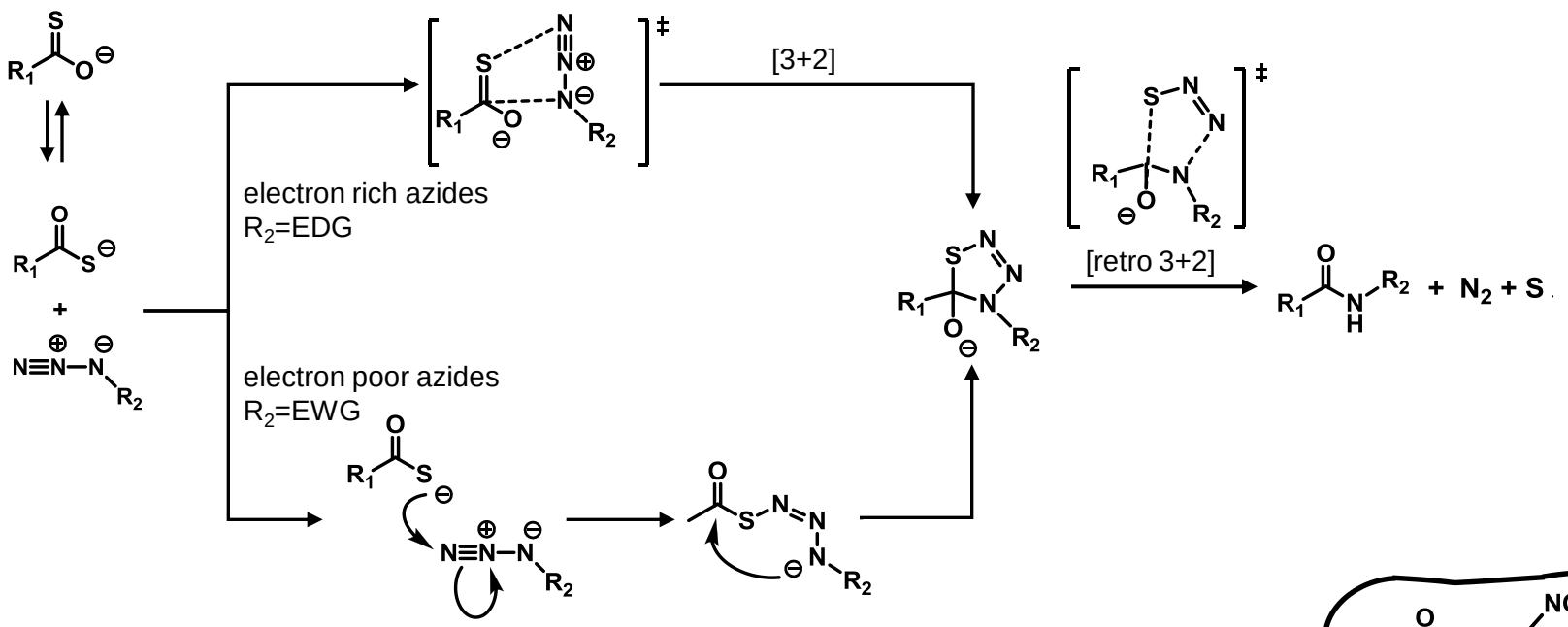
K_D = 300 μM

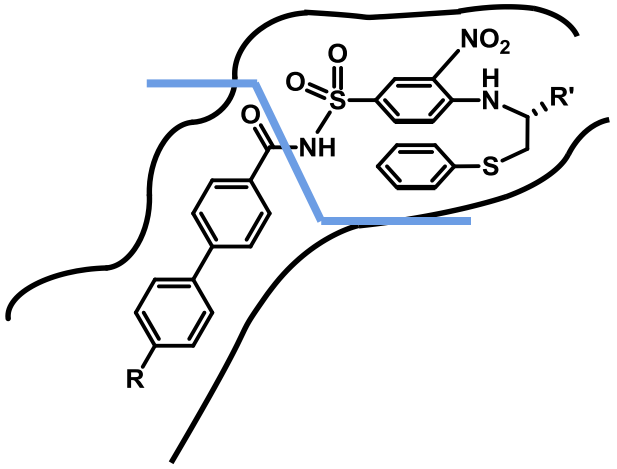


K_i = 19 nM



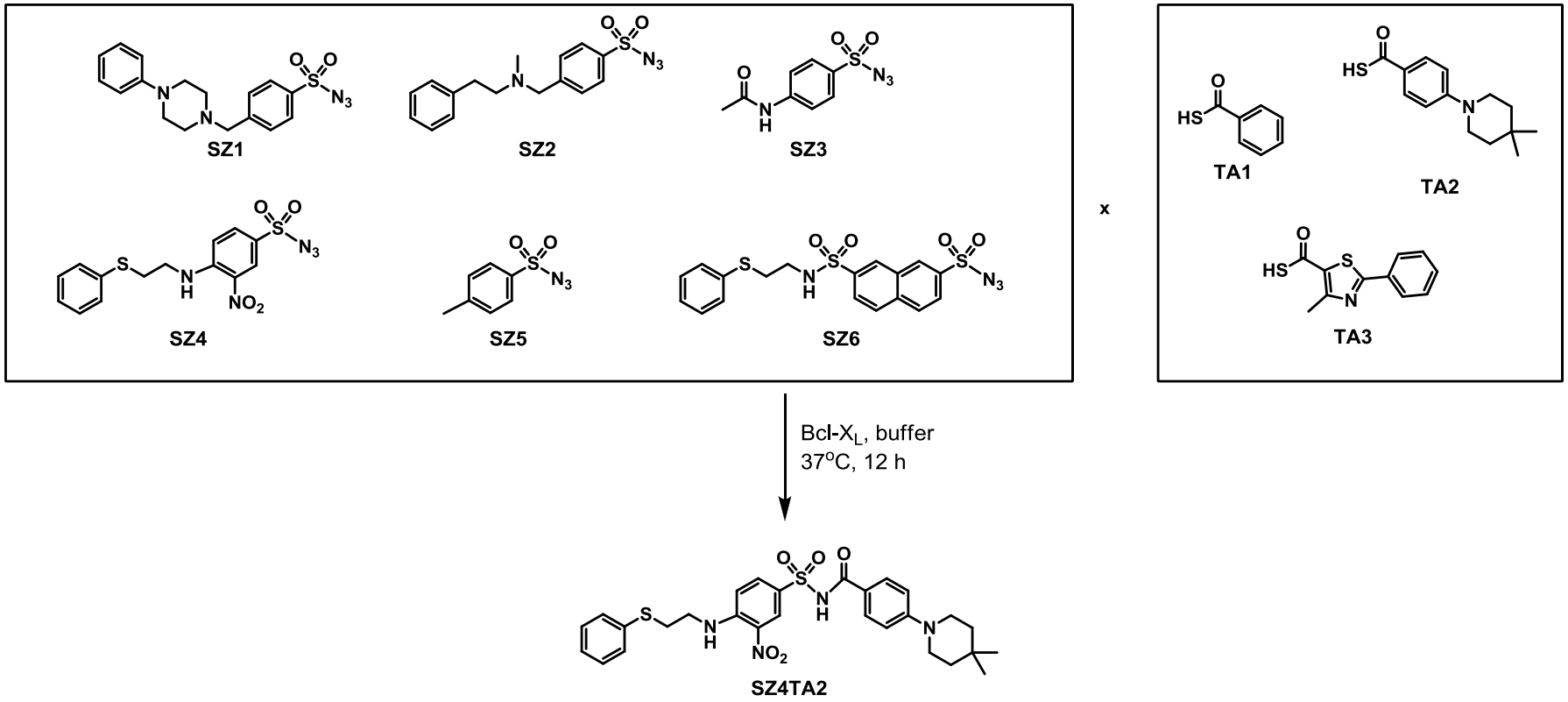
Amidation Reaction





Ning Shangguan, Sreenivas Katukojvala, Rachel Greenberg, Lawrence J. Williams, *J. Am. Chem. Soc.* **2003**, *125*, 7754-7755; Robert V. Kolakowski, Ning Shangguan, Ronald R. Sauers, Lawrence J. Williams, *J. Am. Chem. Soc.* **2006**, *128*, 5695-5702.

Proof of Concept: Bcl-X_L-templated Reactions

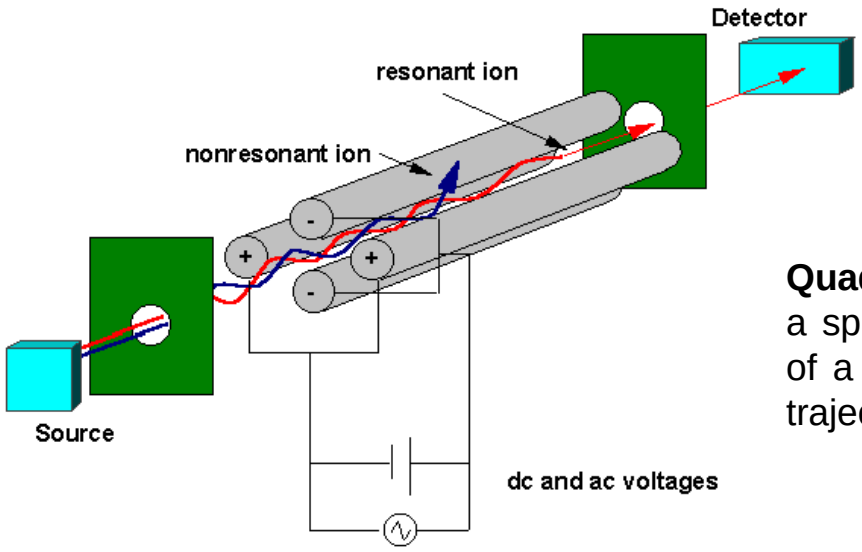


Incubation conditions: 2 μM Bcl-X_L (phosphate buffer, pH=7.4)
20 μM sulfonylazide and 20 μM thioacid building blocks
binary mixtures, 37°C, 12 h
LC/MS-SIM analysis

9 building block reagents → 18 combinations → 1 TGS hit compound

Xiangdong Hu, Jiazhi Sun, Hong-Gang Wang, Roman Manetsch, *J. Am. Chem. Soc.* **2008**, 130(42), 13820-13821

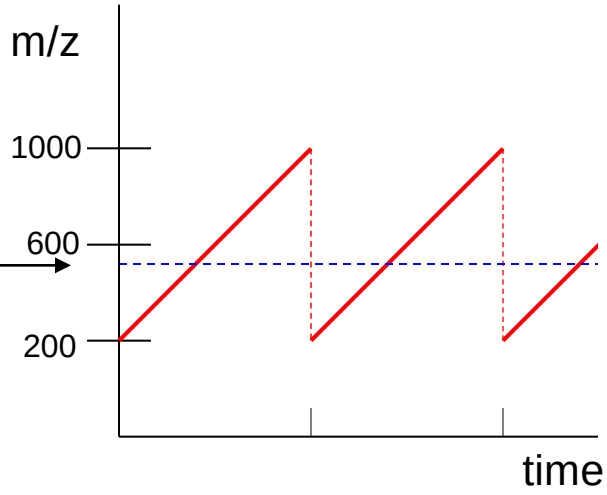
Selected Ion Monitoring *versus* Scan Mode



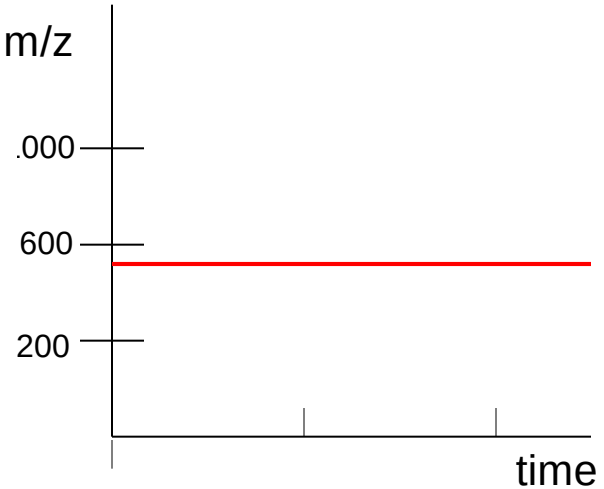
Quadrupole Mass Analyzer: at a specific value of voltage, ions of a particular m/z follow stable trajectory to detector.

SZ4TA2
$[M+H]^+$=569

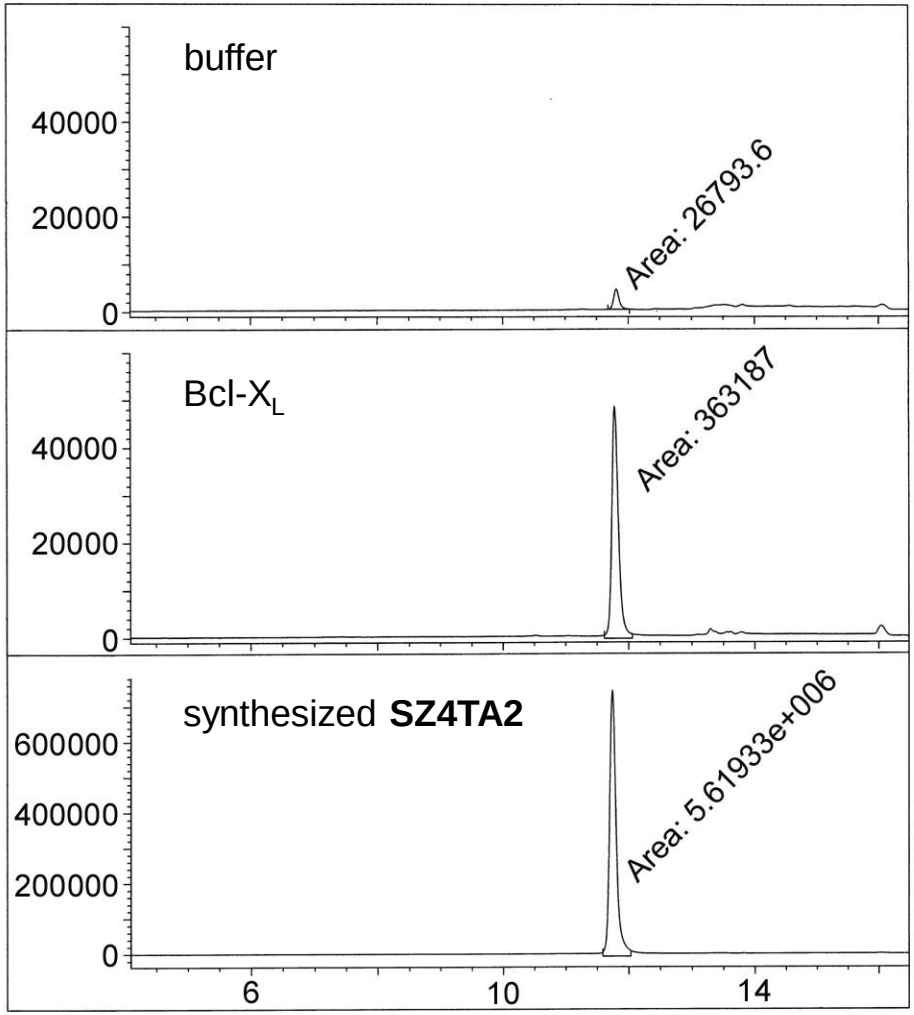
Scan Mode

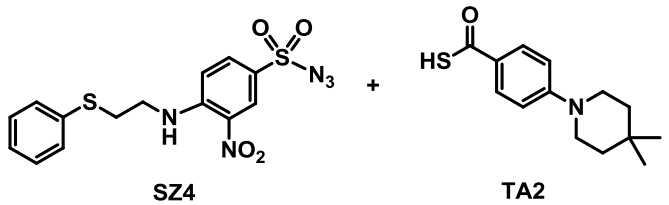


Selected Ion Mode



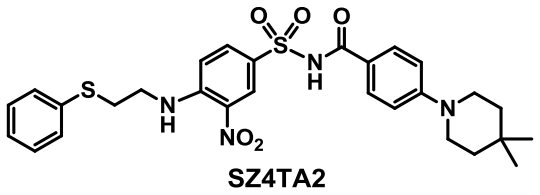
LC/MS-SIM Analysis





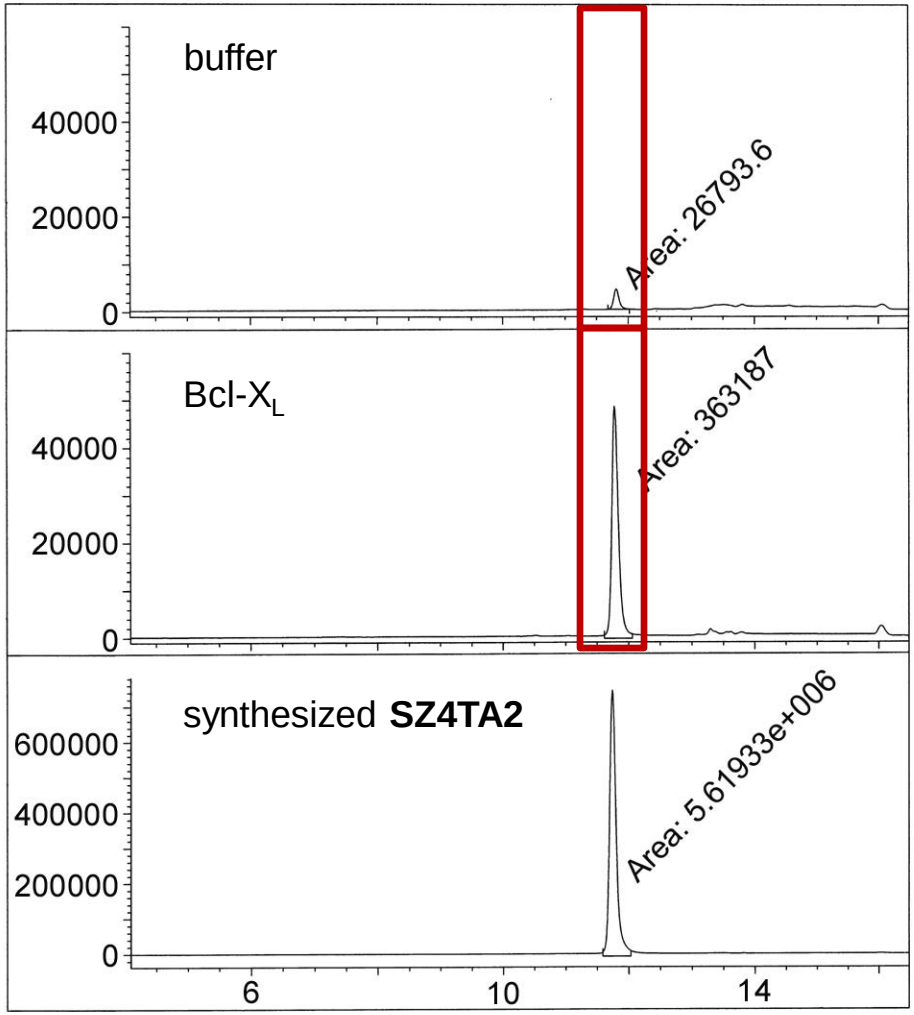
2 μM Bcl-X_L or buffer alone (phosphate buffer, pH=7.4)

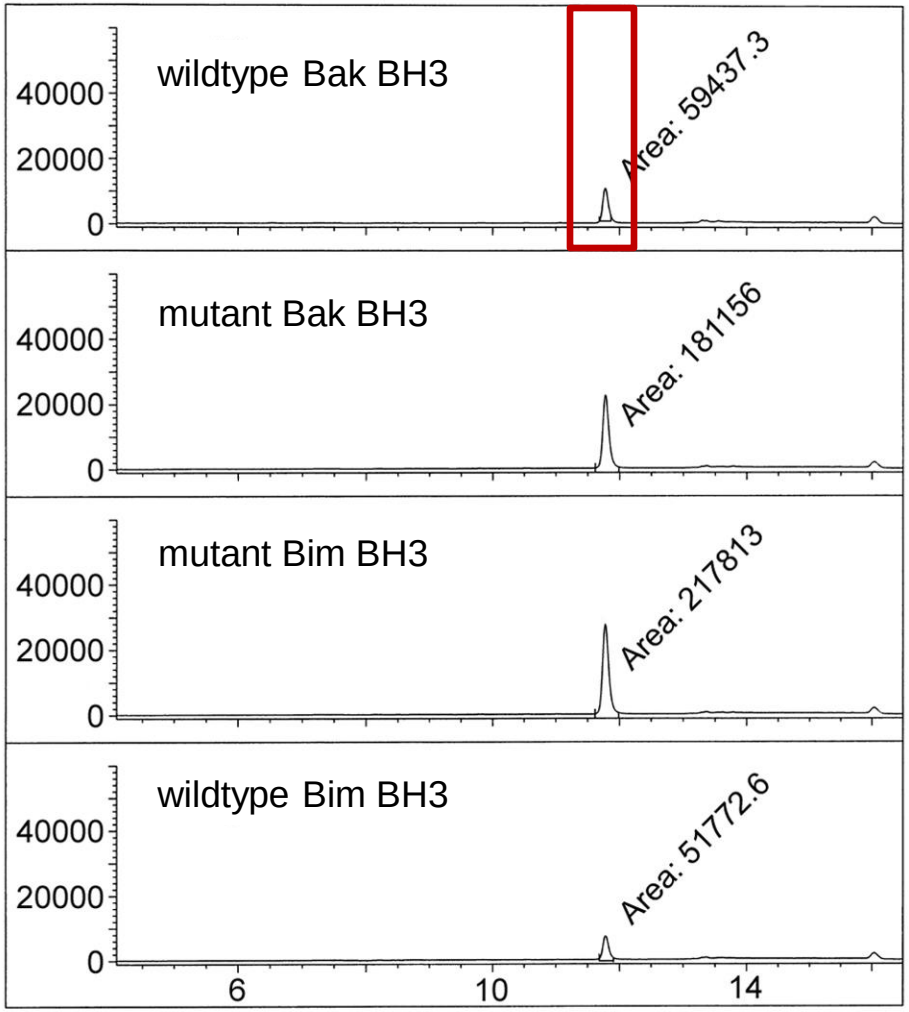
20 μM building blocks
96-well plate, 37 C, 6-12 h
LC/MS-SIM analysis



Xiangdong Hu, Jiazhi Sun, Hong-Gang Wang, Roman Manetsch, *J. Am. Chem. Soc.* **2008**, 130(42), 13820-13821

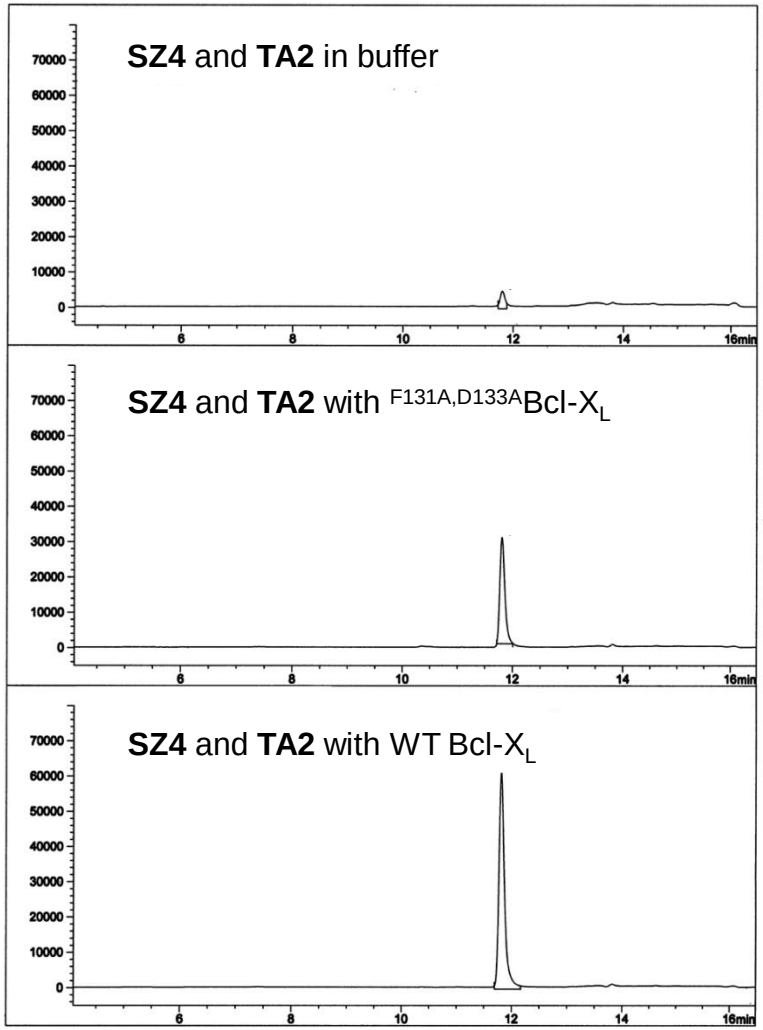
Control Incubations: Competition with Bim or Bak

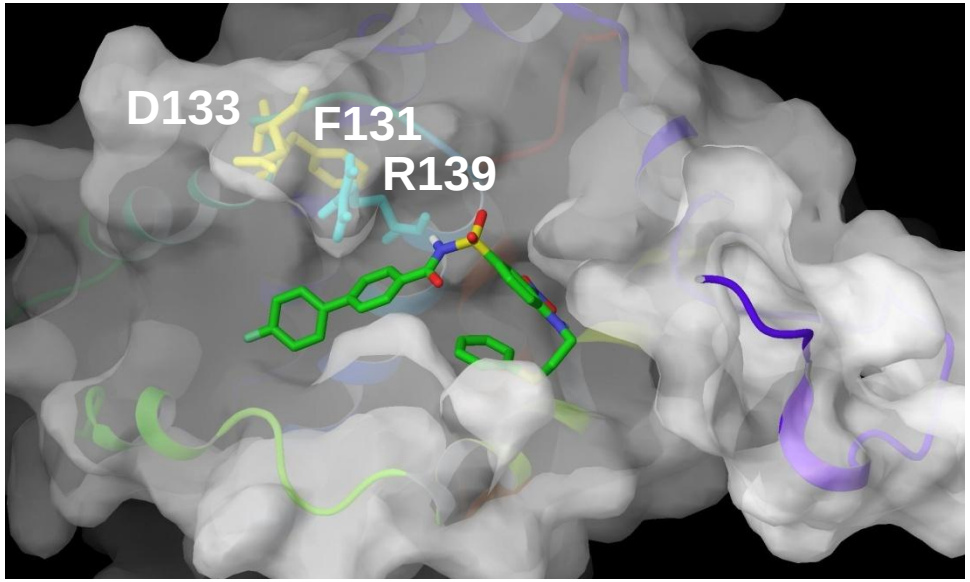




Xiangdong Hu, Jiazhi Sun, Hong-Gang Wang, Roman Manetsch, *J. Am. Chem. Soc.* **2008**, 130(42), 13820-13821

Mutants: Phe131, Asp133 or Arg139 substituted by Ala



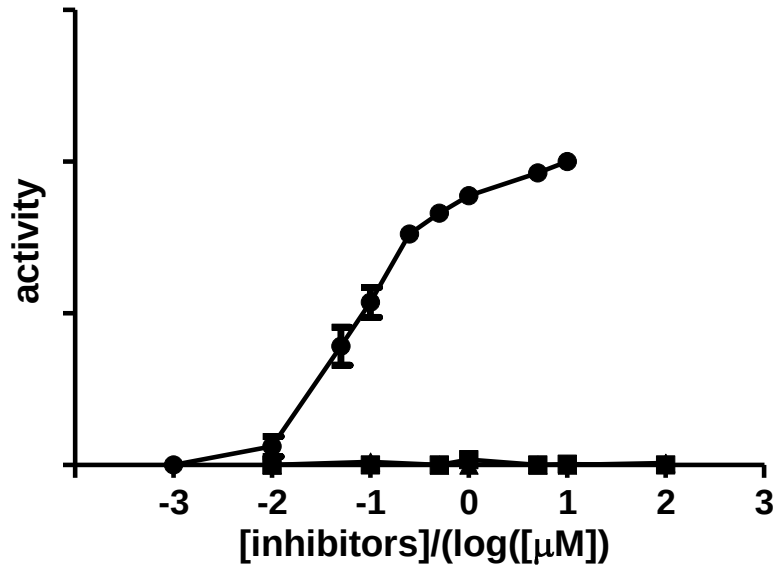


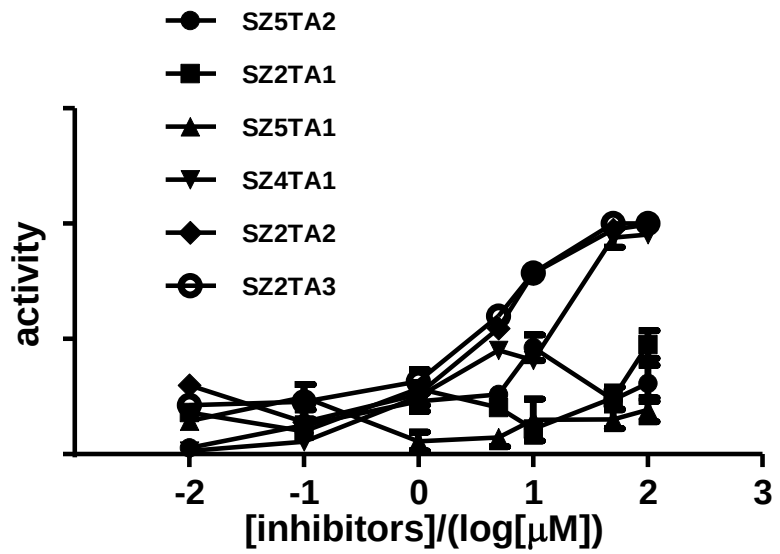
Incubations of **SZ4** and **TA2** with WT or mutant Bcl-X_L and corresponding control incubations with Bak or Bim BH3 peptides.

Entry	Experiment	PA[#]	% Signal
1	Buffer alone	26,794	7.4
2	WT Bcl-X_L	363,187	100.0
3	WT Bcl-X_L and WT Bak	59,437	16.3
4	WT Bcl-X_L and mutant Bak	181,156	49.8
5	WT Bcl-X_L and WT Bim	51,773	14.3
6	WT Bcl-X_L and mutant Bim	217,813	59.9
7	^{F131A,D133A}Bcl-X_L	157,059	43.2
8	^{R139A}Bcl-X_L	95,154	26.2

[#] PA = Peak Area

Binding: Fluorescence Polarization Assay



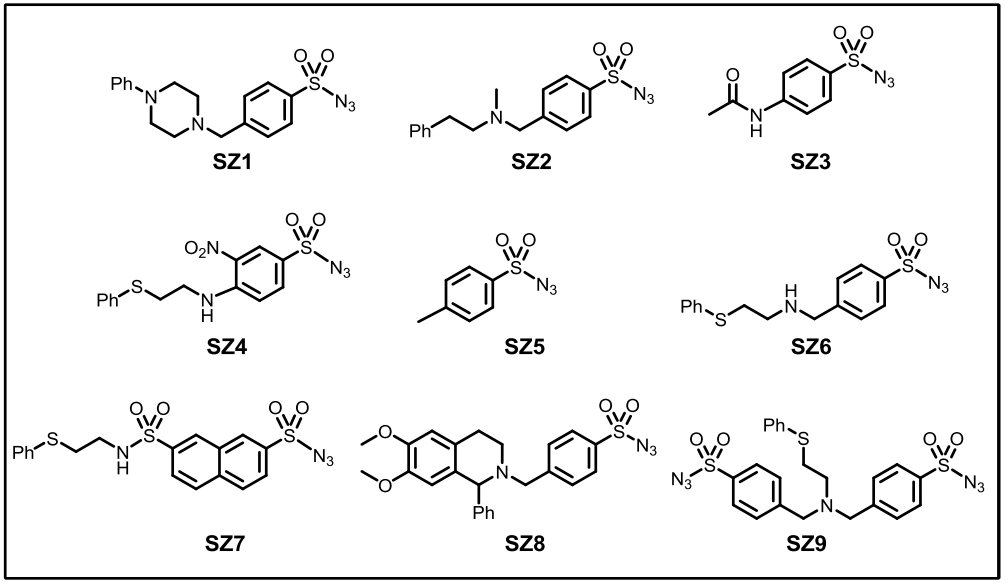


Compound	IC_{50} (FP)
SZ4TA2	78.8 6.7 nM
SZ4	>100 μM
TA2	>100 μM
SZ5TA2	>100 μM
SZ2TA1	>100 μM
SZ5TA1	>100 μM
SZ4TA1	38.9 8.4 μM
SZ2TA2	5.19 1.96 μM
SZ2TA3	5.34 4.24 μM

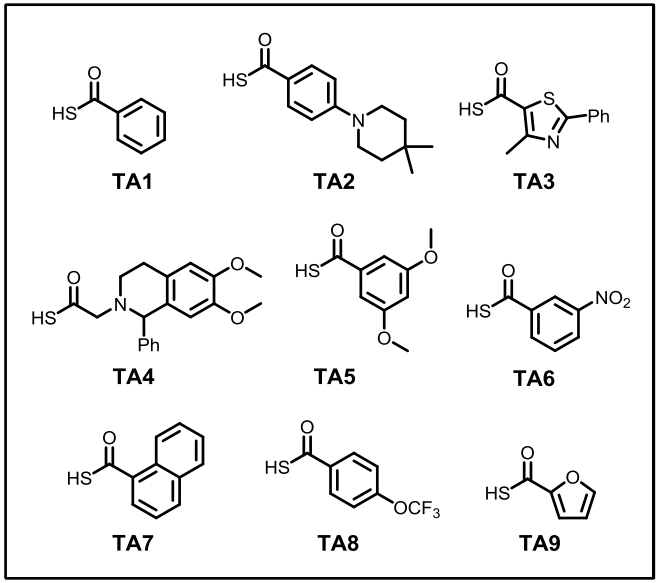
Xiangdong Hu, Jiazhi Sun, Hong-Gang Wang, Roman Manetsch, *J. Am. Chem. Soc.* **2008**, 130(42), 13820-13821

Bcl-X_L-Templated Reactions and Screening

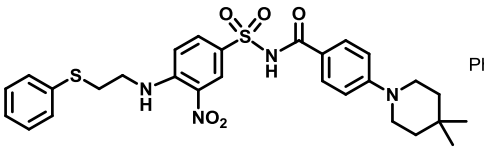
Total 18 reactive building blocks: 9 sulfonyl azides and 9 thio acids leading to 81 potential amides (9 x 9= 81)



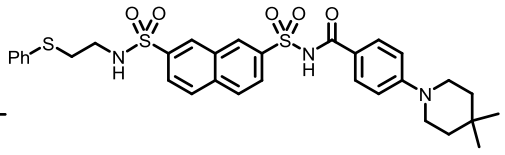
x



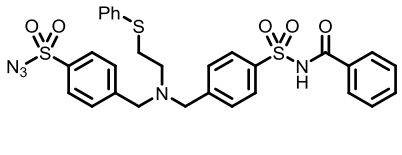
Bcl-X_L, phosphate buffer pH=7.4,
38°C, 6 hours, LC/MS-SIM


SZ4TA2

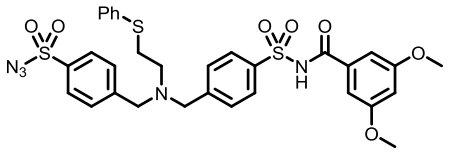
IC_{50} = 106 nM
Reported : K_i = 19 nM


SZ7TA2

IC_{50} = 28.6 µM

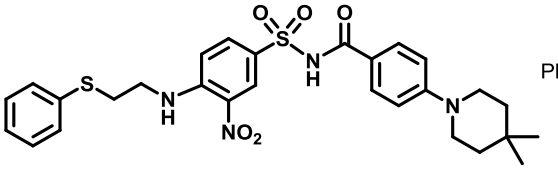

SZ9TA1

IC_{50} = 28.8 µM

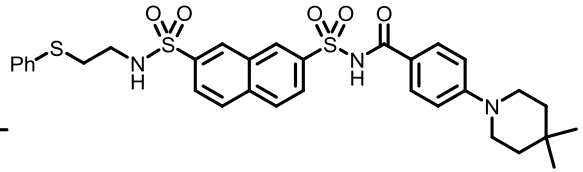

SZ9TA5

IC_{50} = 37.2 µM

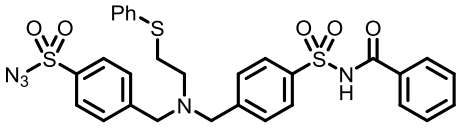
Are TGS Hit Compounds the Most Potent Compounds?



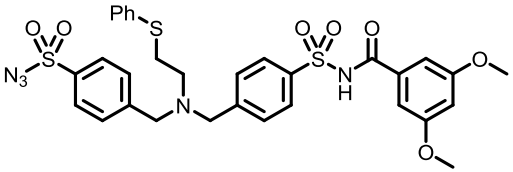
SZ4TA2



SZ7TA2



SZ9TA1



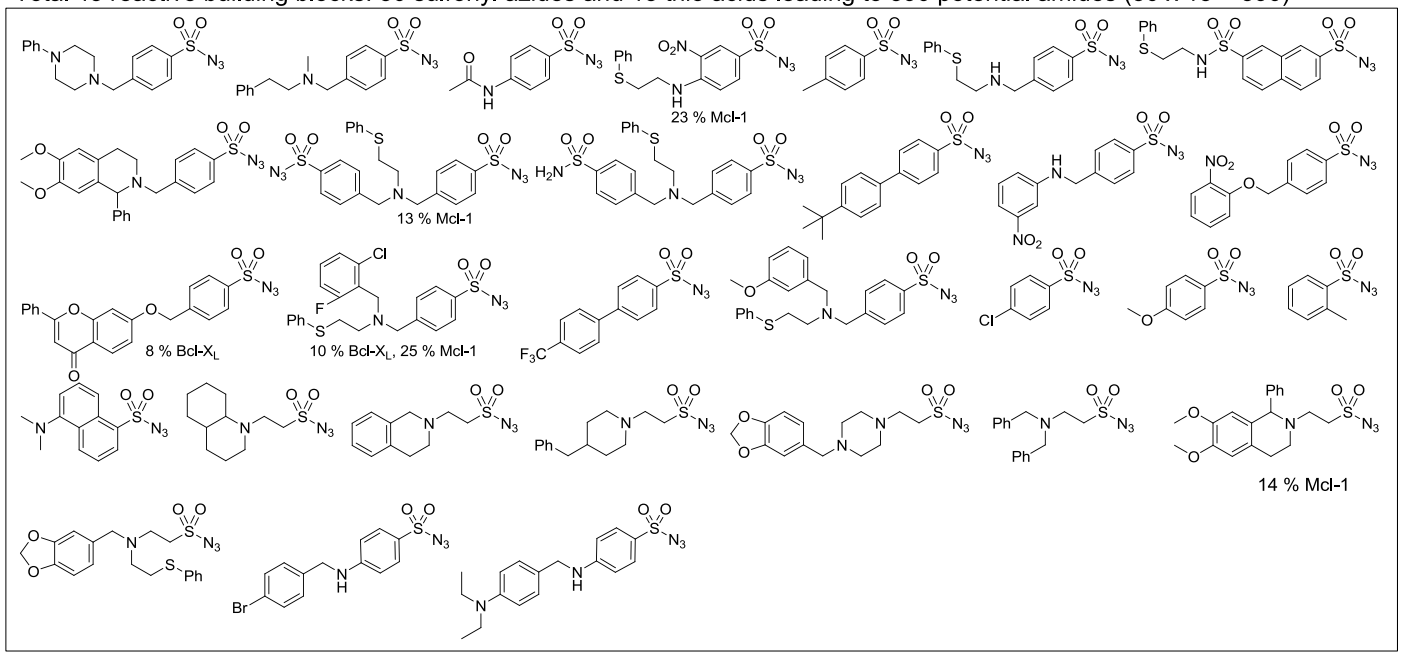
SZ9TA5

Fragments	SZ1	SZ2	SZ3	SZ4	SZ5	SZ6	SZ7	SZ8	SZ9
TA1	nd	2	0	14	29	nd	nd	19	80
TA2	nd	9	nd	100	28	26	76	nd	38
TA3	6	7	nd	nd	nd	nd	nd	30	22
TA4	nd	25	nd	nd	nd	nd	nd	8	nd
TA5	5	nd	nd	nd	0	nd	15	11	60
TA6	4	nd	0	nd	0	nd	20	nd	46
TA7	nd	nd	0	nd	nd	nd	47	30	nd
TA8	3	nd	0	nd	nd	nd	nd	38	nd
TA9	nd	nd	0	nd	1	nd	nd	24	nd

% inhibition at 50 µM acylsulfonamide concentration; 37 compounds synthesized and tested

Parallel Kinetic TGS Screening of Multiple PPIs

Total 43 reactive building blocks: 30 sulfonyl azides and 13 thio acids leading to 390 potential amides (30 x 13 = 390)



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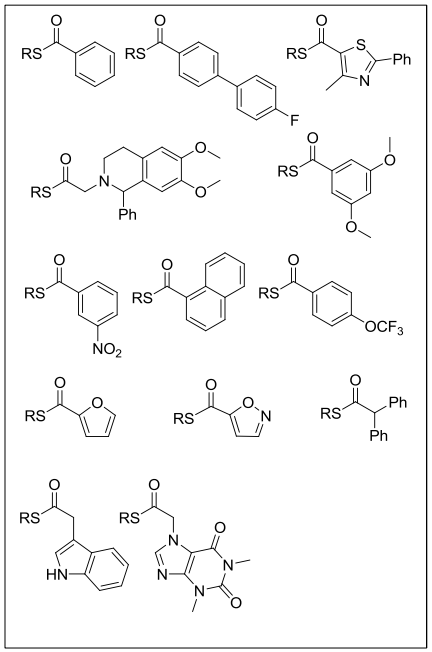
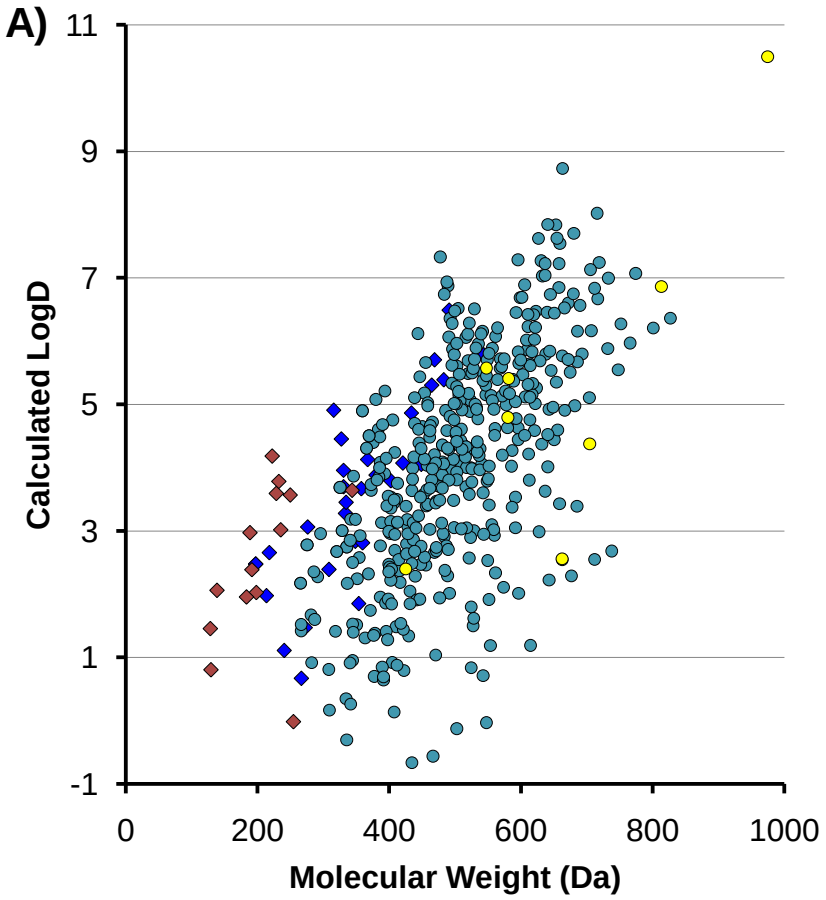
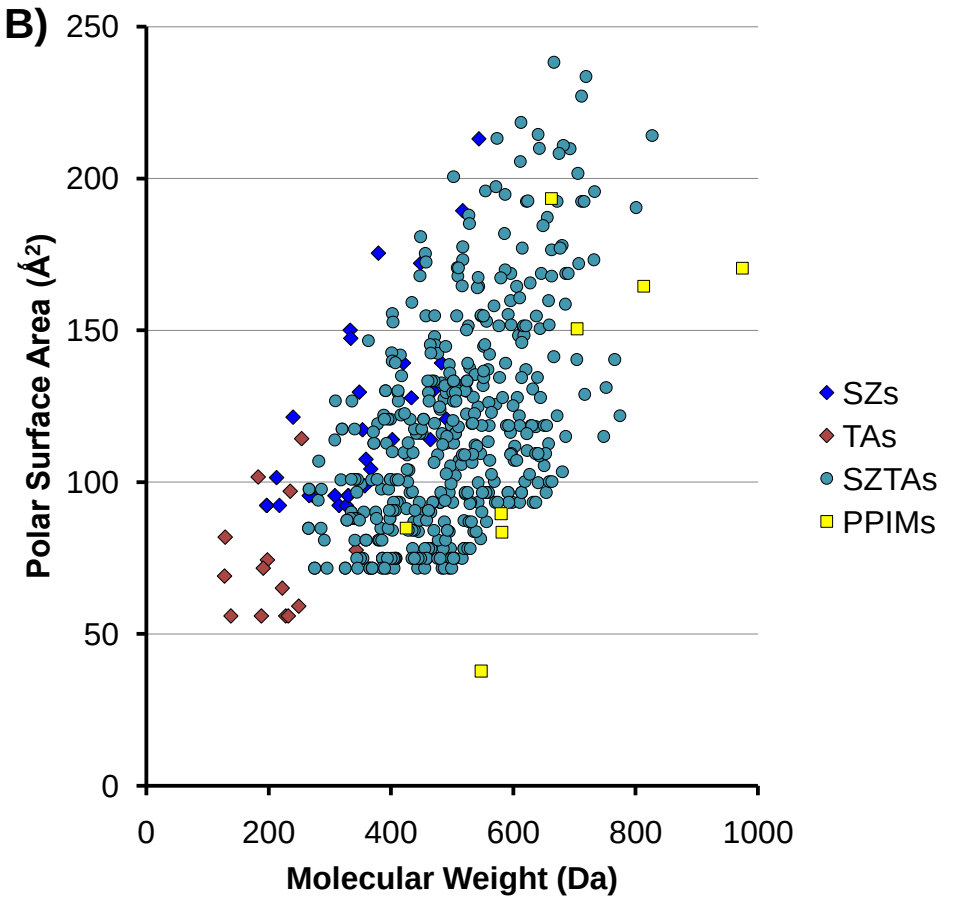
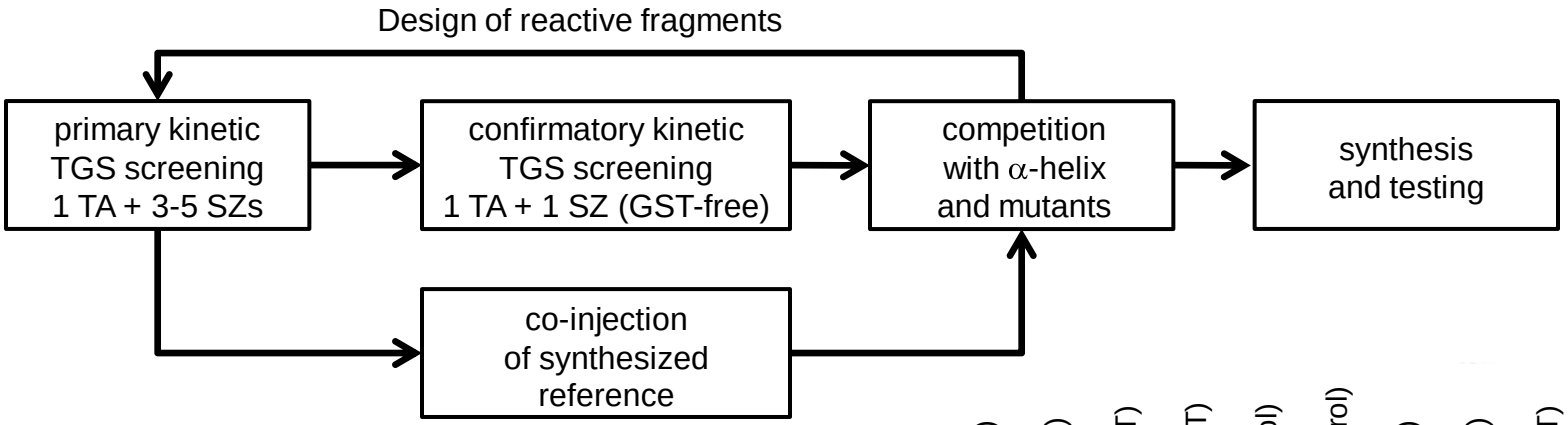


Building Blocks and Acylsulfonamides





Parallel Kinetic TGS Screening of Multiple PPIs

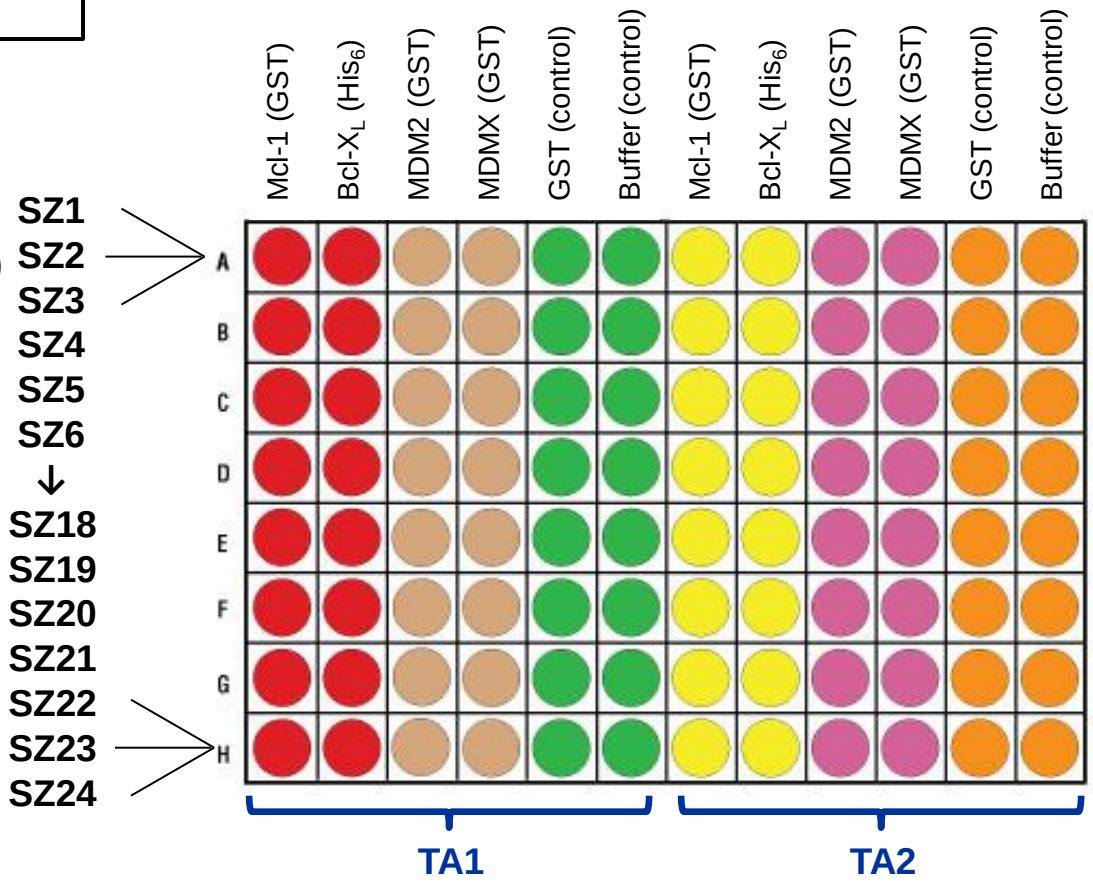


Throughput 4 PPIs

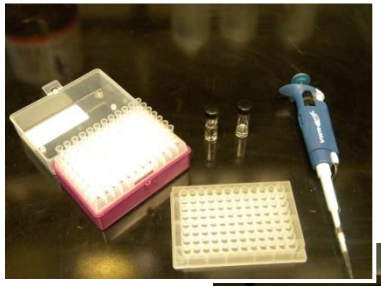
- 4 protein targets + 2 controls
- 48 – 80 combinations/(day·target)
- 8 minutes LC/MS runs

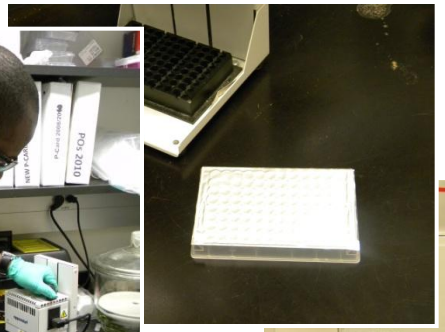
Throughput 1 PPI

- 144 – 240 combinations/day



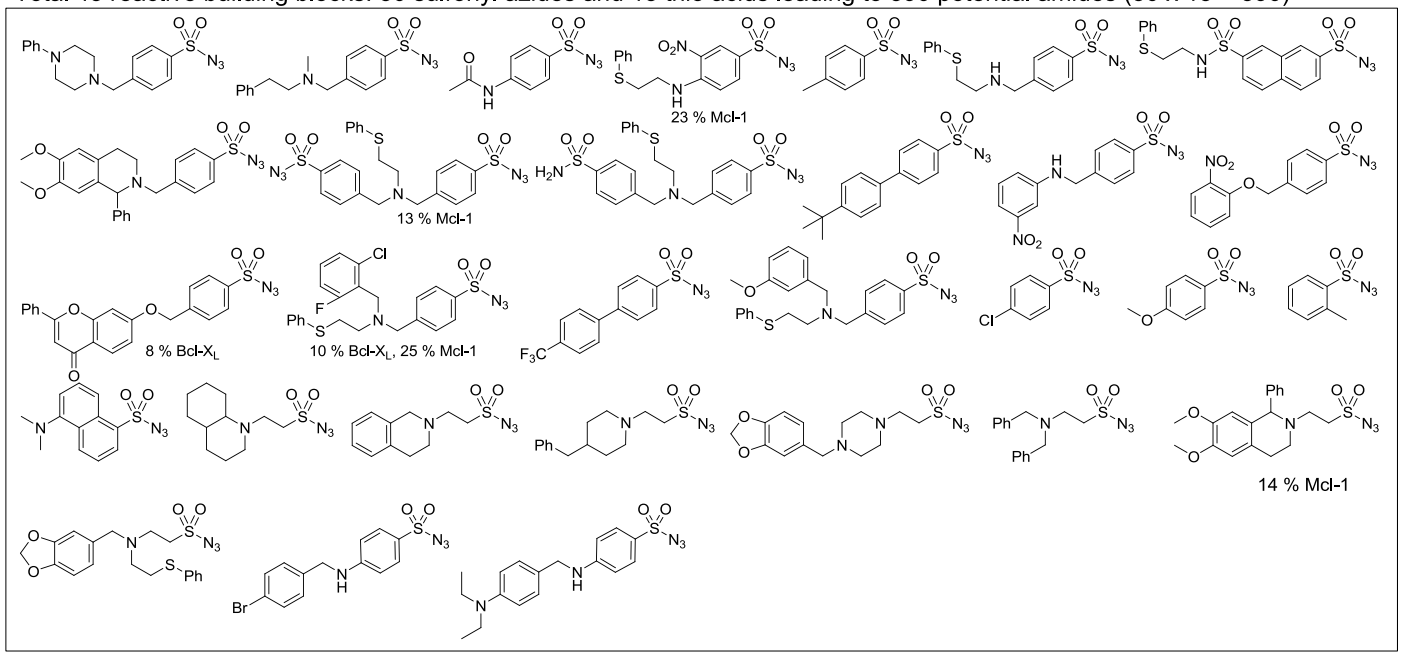
Parallel Kinetic TGS Screening of Multiple PPIs





Parallel Kinetic TGS Screening: Example Mcl-1

Total 43 reactive building blocks: 30 sulfonyl azides and 13 thio acids leading to 390 potential amides (30 x 13 = 390)



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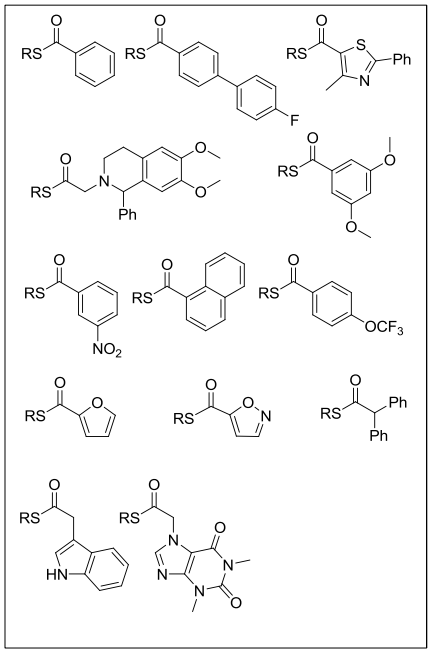

Incubation conditions:
- 8 μM protein (phosphate buffer, pH=7.4)
- 20 μM sulfonylazide and 20 μM thioacid building blocks
- Mixtures of 1 thio acid and 3-5 azides, 37 C, 12 h
- LC/MS-SIM analysis

Example: ± 500 combinations → 13 kinetic TGS hits for Mcl-1 → 1 SZ class

Conclusions

- Amidation between thioacids and sulfonylazides can be utilized for kinetic TGS
- Control incubations to confirm that kinetic TGS occurs at the hot spots of Bcl-X_L, Mcl-1, or any other PPIs
- Kinetic TGS hit acylsulfonamides are more potent compared to acylsulfonamides not discovered through kinetic TGS
- Straightforward and rapid “in-well” activation of thioesters to yield thioacids
- Kinetic TGS screening lead to the discovery of selective acylsulfonylamide inhibitors targeting Mcl-1 over Bcl-X_L

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