STRUCTURE-GUIDED RESCAFFOLDING OF SELECTIVE ANTAGONISTS OF BCL-X_L

Michael Koehler, Philippe Bergeron, Edna Choo, Kevin Lau, Chudi Ndubaku, Danette Dudley, Paul Gibbons, Brad Sleebs, Carl Rye, George Nikolakopoulos, Chinh Bui, Sanji Kulasegaram, Wilhelmus Kersten, Brian Smith, Peter Czabotar, Peter Colman, David Huang, Jonathan B. Baell, Keith Watson, Lisa Hasvold, Zhi-fu Tao, Le Wang, Andrew Souers, Steven Elmore, John Flygare, Wayne Fairbrother, Guillaume Lessene

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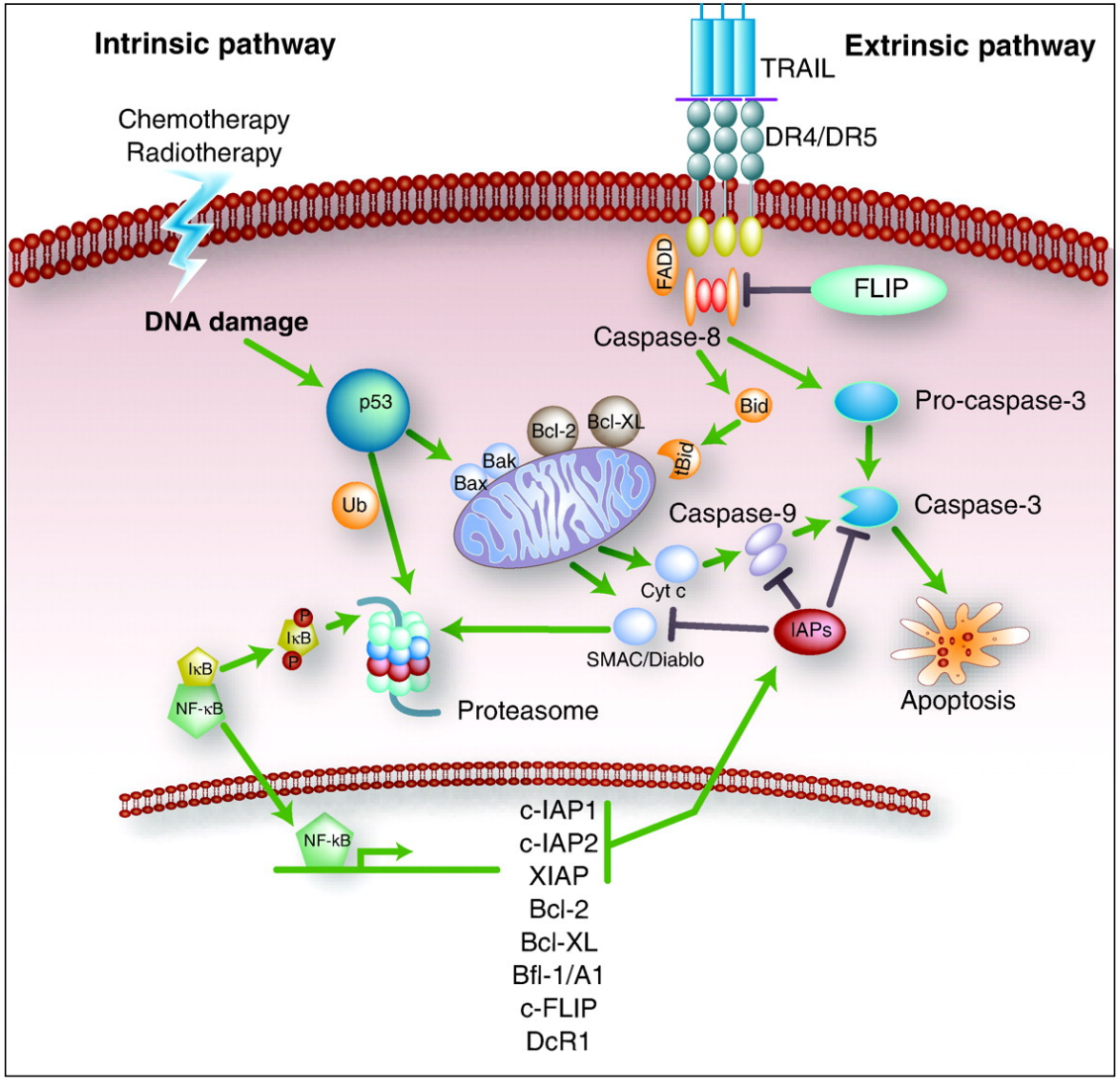
CURRENT LITERATURE

4/12/2014

APOPTOSIS

- Extrinsic pathway
  - Pro-apoptotic proteins bind at surface
  - Initiate caspase cascade
- Intrinsic pathway
  - Cellular stress
  - Cellular pro-apoptotic proteins produced/ accumulate
  - Interact with pro-survival proteins/ apoptotic effectors
  - Permeabilize mitochondrial membrane
  - Initiate caspase cascade



CCR Molecular Pathways

Clin. Cancer Res. **2006**, 12, 2390-2393.

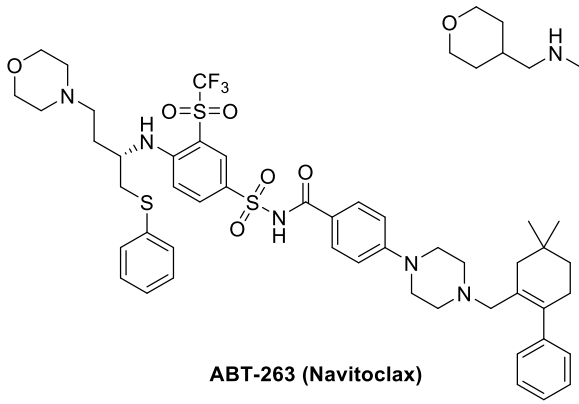
INHIBITORS OF APOPTOSIS

Extrinsic pathway

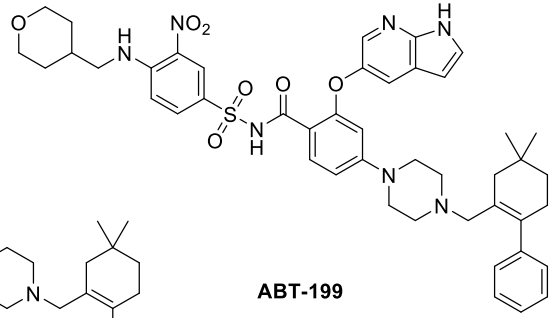
- Systemic delivery of Apo2L/TRAIL or agonistic Apo2L/TRAIL death receptor antibodies
- Typically in conjunction with chemotherapeutics
  - Synergy seen with Apo2L/TRAIL and cytotoxic agents

Intrinsic pathway

- Mainly targeting overexpressed pro-survival proteins and inhibitor of apoptosis (IAP) proteins
- Generally target protein-protein interactions
- Designed to be BH3 mimetics



ABT-263 (Navitoclax)



ABT-199

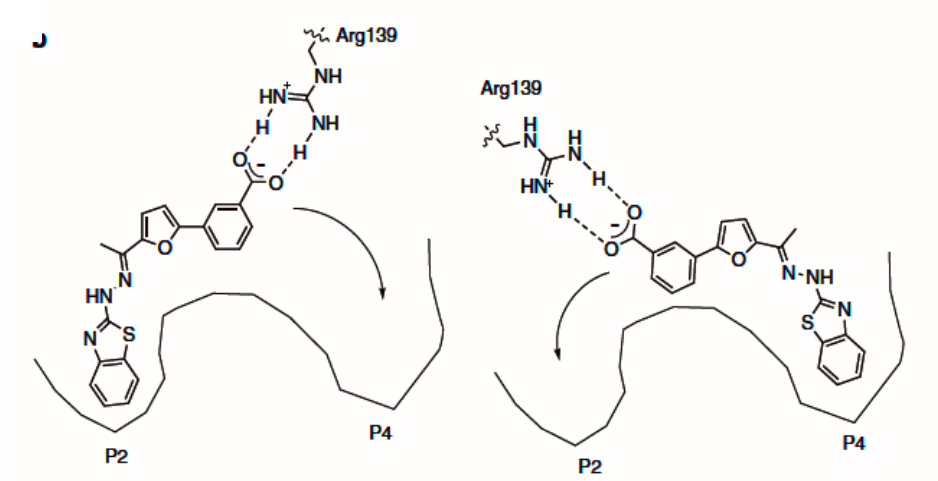
Cancer Immunol. Immunother. **2011**, 60, 1173–1180.

WHY BCL-X_L

- BCL-X_L is a pro-survival protein that is sequestered by BH3-only proteins which causes permeabilization of the mitochondrial membrane
- Strongly implicated as a chemoresistance factor
  - However BH3 protein binding site is a shallow grove, potentially difficult to selectively target
- Pan pro-survival protein inhibition has the potential for undesirable effects
  - They are essential in various cell types under normal conditions
- Selective inhibitors (ABT-199) of other pro-survival proteins (BCL-2) have been shown to be effective against chronic lymphocytic leukemia (CCL)
  - Without ***as many*** side effects as are observed for pan inhibitors
- Believed BCL-X_L selective inhibitors would lack undesired effects on BCL-2 hematopoetic cells

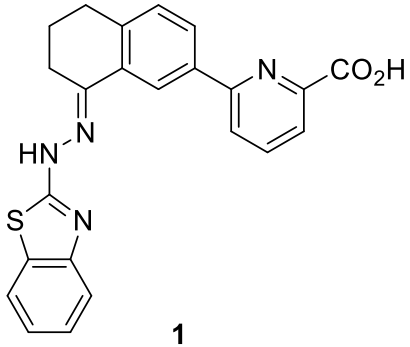
INITIAL WORK

- HTS of ~100,000 compounds
- Discovered a series of benzothiazole-hydrazone core compounds that were active against BCL-X_L
- BH3 proteins are known to have important associations with P2 and P4 in BCL-2 related proteins
- Believed that the small lead compound was only able to occupy one pocket at a time
  - Designed compounds to occupy both and elaborated based on co-crystal structure with extended analog



Nat. Chem, Biol. **2013**, **9**, 390-397.

INITIAL WORK



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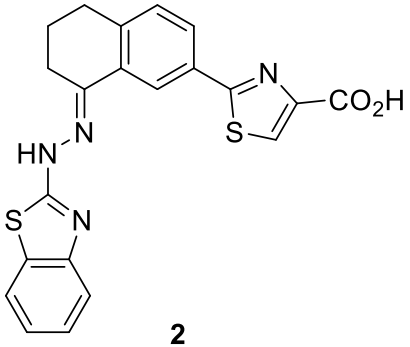

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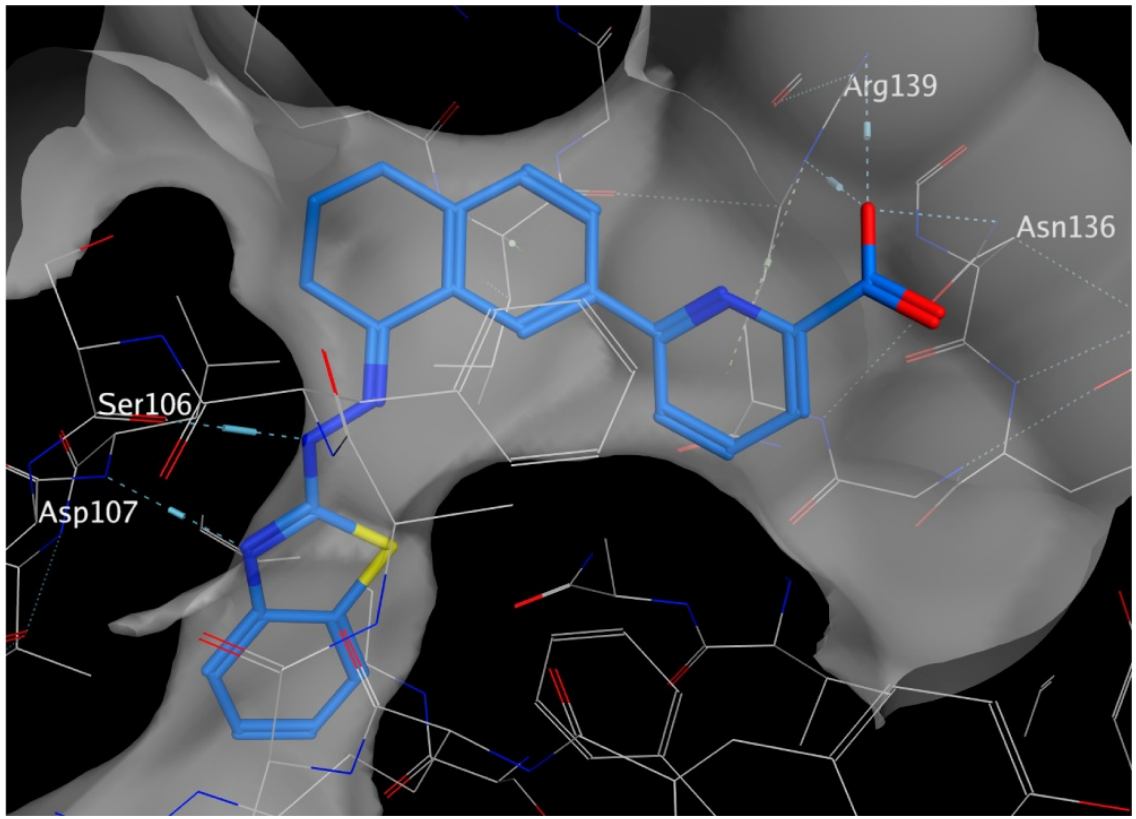
	BCL-X_L IC_{50} (μM)	BCL-2 IC_{50} (μM)	Rat CL (mL/min·kg) (1 mg/kg IV)	Rat F% (5 mg/kg PO)
1	0.020	>10	9.6	8
2	0.013	5	44	4

TOXICITY OF HYDROZONES

- Not necessarily definitively toxic
- Most companies prefer to eliminate hydrazone early on to prevent issues of toxicity after significant time and money are invested

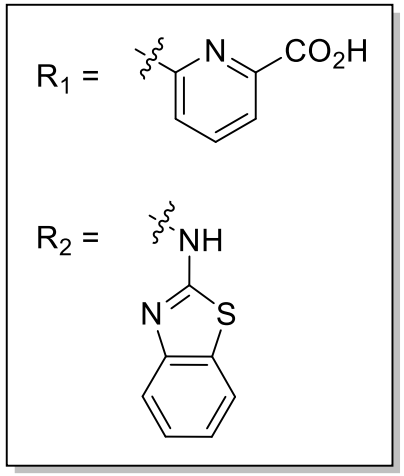
- Known to hydrolyze and release hydrazines
  - Metabolically converted to diazine, diazonium, and radical metabolites
  - Can then react to alkylate DNA and proteins

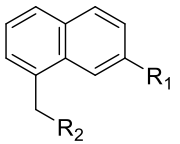
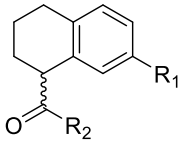
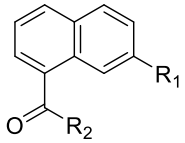
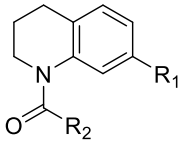
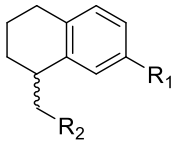
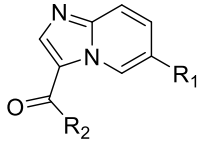
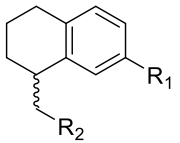
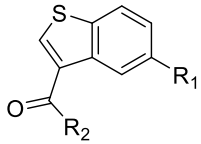
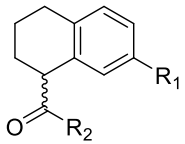
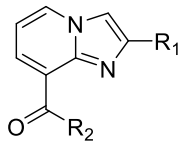
WHERE TO BEGIN



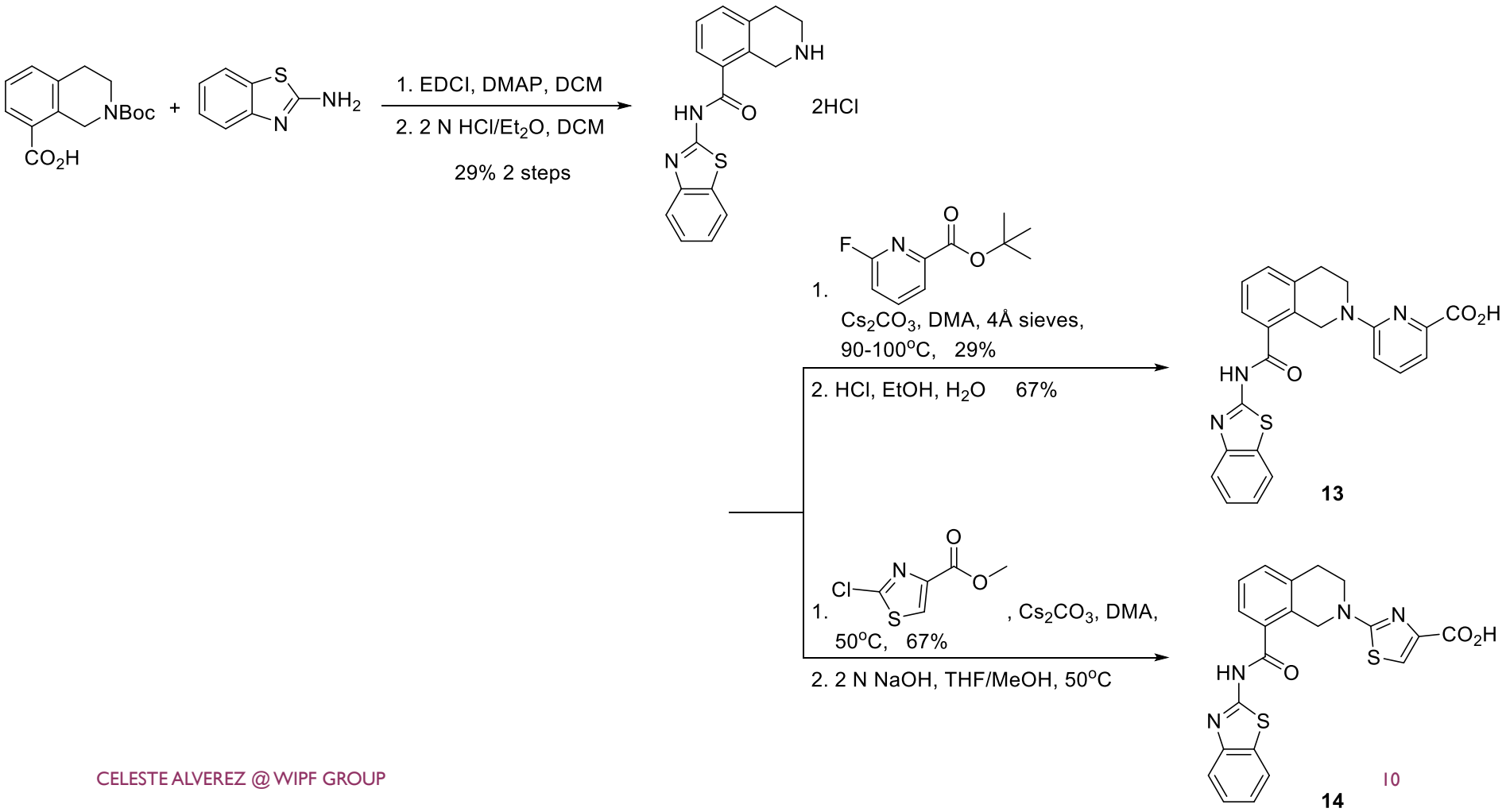
Hydrazone **I** co-crystallized with BCL-X_L

CORE OPTIMIZATION



	Core	BCL-X_L IC_{50} (μM)		Core	BCL-X_L IC_{50} (μM)
3		>20	**6b**		>20
4		1.0	**7**		0.72
5a		>20	**8**		5.3
5b		9.2	**9**		5.1
6a		9.6	**10**		>100

GENERAL SYNTHESIS



P4 TARGETING EXTENSIONS

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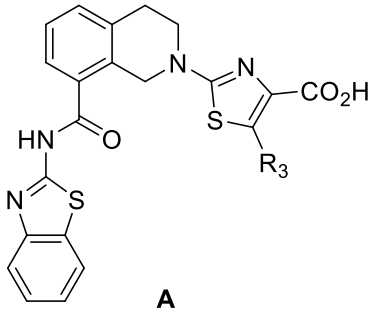
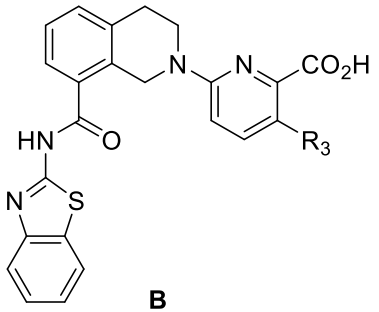
	Scaffold	R_3	BCL-X_L IC_{50} (nM)	MEF mcl-$1^{-/-}$ EC_{50} (μM)
13	**B**	H	270	2.4
14	**A**	H	91	n.d.
15	**B**	$(CH_2)_2Ph$	610	-
16	**B**	$(CH_2)_3Ph$	32	-
17	**B**	$(CH_2)_3OPh$	16	-
18	**B**	$(CH_2)_4OPh$	43	-
19	**B**	CH=CHPh	90	-
20	**B**	$CH=CHCH_2Ph$	4	-
21	**A**	$(CH_2)_2OPh$	86	-
22	**A**	$(CH_2)_3OPh$	1	0.014
23	**A**	$(CH_2)_4OPh$	7	-



A



B

SELECTIVITY

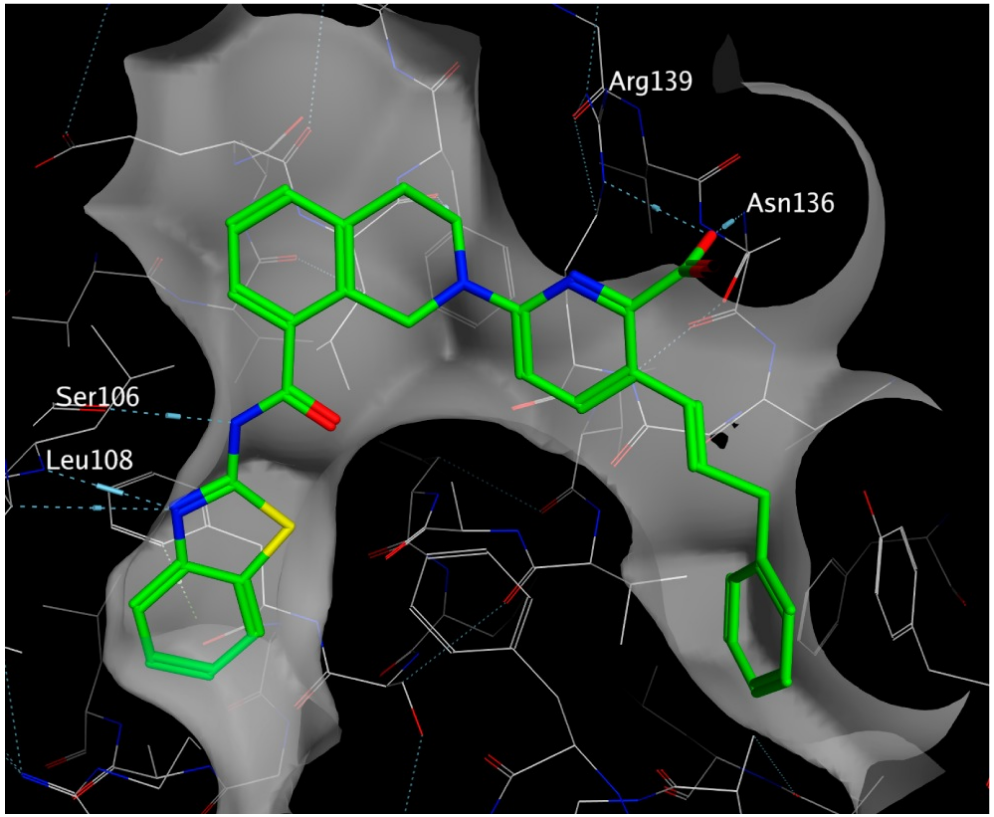
	Surface Plasmon Resonance K_d (μM)			
Compound	BCL-X_L	BCL-2	BCL-w	Mcl-1
13	0.038	>20	>20	>20
14	0.010	9.2	7.8	>20
22	<0.005	4.4	0.062	14.4

PK ANALYSIS

Rat PK data

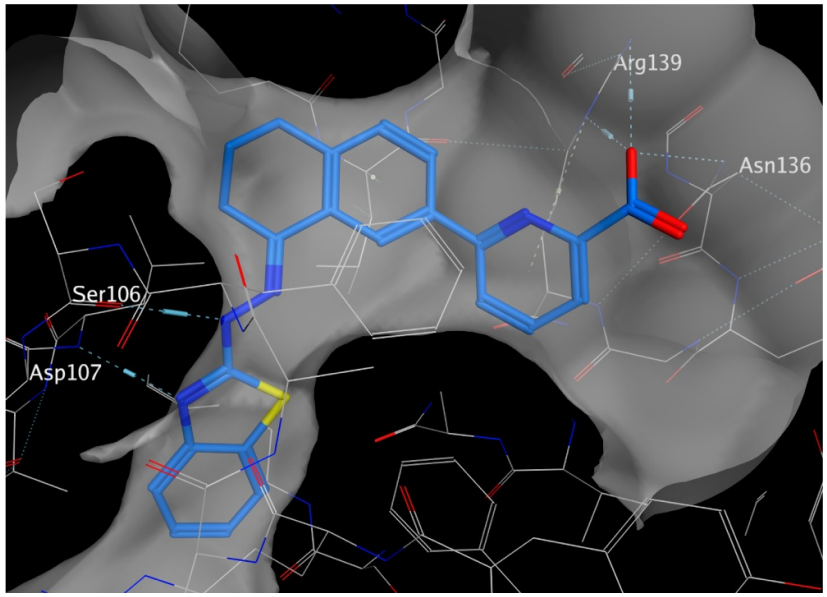
	IV (1 mg/kg)			PO (5 mg/kg)
Compound	CL_p (mL/min·kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	F%
13	0.20	0.12	8.3	60
14	0.47	0.16	6.0	16
22	7.4	0.31	3.6	0.2

COMPARISON TO LEAD

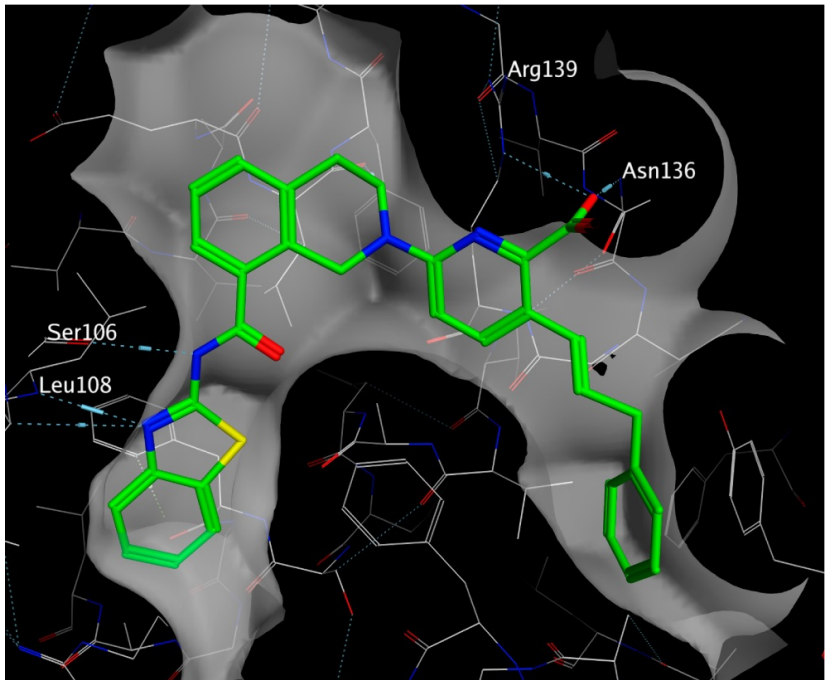


Tetrahydroisoquinoline **20** co-crystallized with BCL-X_L

COMPARISON TO LEAD



Hydrazone **1** co-crystallized with BCL-X_L



Tetrahydroisoquinoline **20** co-crystallized with BCL-X_L

CONCLUSIONS/FUTURE DIRECTIONS

- Using a co-crystal structure with known interactions the authors were able to design potential cores to maintain desired interactions
- Synthesis and subsequent testing lead to a series of novel inhibitors which removed the potentially toxic hydrazone core
  - Maintaining efficacy and PK properties
- Were able to take an previously optimized inhibitor and through core hopping mitigate a potential toxicity problem while maintaining efficacy

- Further explore thiazole 5 position for improving potency and increase PK properties
- Replace thiazole S with O to try to improve PK
  - Find a balance between **22**'s high potency and **13**'s desirable PK profile