The Importance of Being Me: Magic Methyls, Methyltransferase Inhibitors, and the Discovery of Tazemetostat

Kuntz, K. W.; Campbell, J. E.; Keilhack, H.; Pollock, R. M.; Knutson, S. K.; Porter-Scott, M.; Richon, V. M.; Sneeringer, C. J.; Wigle, T. J.; Allain, C. J.; Majer, C. R.; Moyer, M. P.; Copeland, R. A.; Chesworth, R.

J. Med. Chem. **2016**, *59*, 1556-1564

Celeste Alverez

Current Literature

March 12, 2016

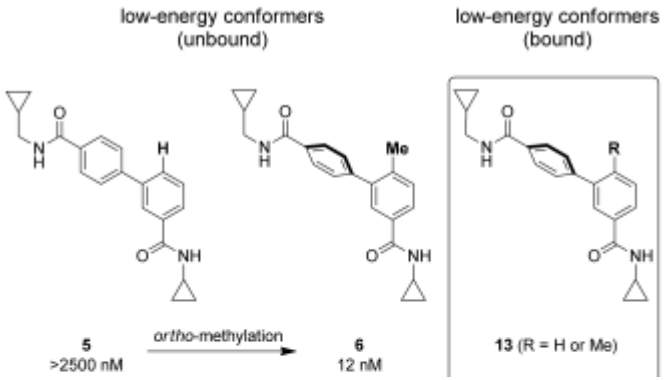
What is the Magic Methyl Effect?

The addition of a methyl group to a bioactive molecule resulting in a increase in potency

- Typically modest, but at times significant

Me can affect solvation, hydrophobic interactions, and sterics (size and conformation)

- Methylation results in decreased solvation of the molecule reducing the energy needed to desolvate to bind to the protein
- If there is space in the binding pocket a methyl group could fit into it resulting in increased interactions and improving potency
- A large proportion of the effect on binding is likely the conformation of the unbound ligand the bound ligand
  - Requiring less energy to adopt the necessary shape for binding increasing potency



Angew. Chem Int. Ed. **2013**, *52*, 12256-12267

Why is Methyl so Magical?

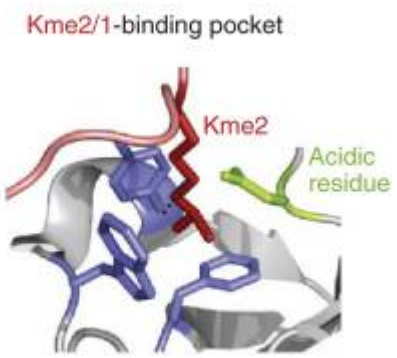
In biology post-translational modifications to the chromatin regulates gene expression

- Histone methylation is a key PTM
  - 0, 1, 2, or 3 Me on K and R of histones

If this system isn't tightly regulated it can result in uncontrolled cell proliferation and cancer

Specificity? (millions to billions of possible states of methylation)

- Formation of a hydrophobic (aromatic) cage at the binding site of the K or R resulting in cation-π interactions
- Selection of mono- or dimethylated substrates over trimethylated due to replacement of a wall of the cage with a negatively charged reside resulting in additional H-bonding and electrostatic interactions



Nat. Struct. Mol. Biol. **2012**, 19, 1218–1227

EZH2

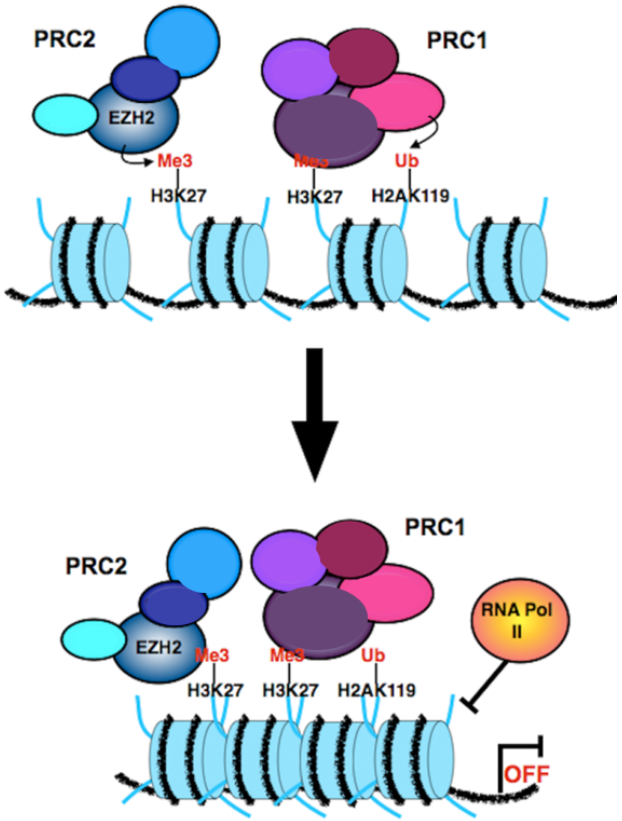
Enhancer of Zeste Homologue 2

The catalytic subunit of PRC2 (polycomb repressive complex 2)

- Responsible for epigenetic regulation of genes

Protein lysine methyltransferase (PKMT)

- Transfers methyl groups from SAM (S-adenosyl methionine) to specific lysines within histones
- Specifically methylates histone 3 at lysine 27 (H3K27)



Adapted from Marchesi, I. and Bagella, L. Role of Enhancer of Zeste Homolog 2 Polycomb Protein and Its Significance in Tumor Progression and Cell Differentiation. In *Chromatin Remodelling;* Radzioch, D., Ed.; InTech. http://www.intechopen.com/books/chromatin-remodelling/role-of-enhancer-of-zeste-homolog-2-polycomb-protein-and-its-significance-in-tumor-progression-and-c

EZH2

Overexpression and mutation have been linked to cancer and are considered oncogenic

WT prefers to methylate H3K27 from 0 → 1 Me

Mutants prefer to methylate 1 → 2 and/or 2 → 3 Me

- H3K27me3 represses gene expression (cancer cells become dependent on this to reduce expression of tumor suppressor genes)

Y646X is known mutation of EZH2

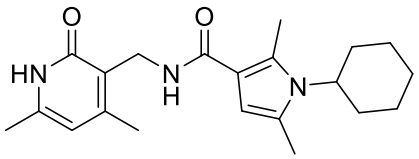
- In related PKMTs, it was shown mutation of Y to F resulted in expansion of substrate to dimethylated K
  - The 2nd Me fit in the space formerly occupied by the OH of the Y accommodating for this additional Me

Targeting EZH2 should result in reduced cellular proliferation, expression of tumor suppressor genes and possibly cell death in cells dependent on hyper-trimethylation

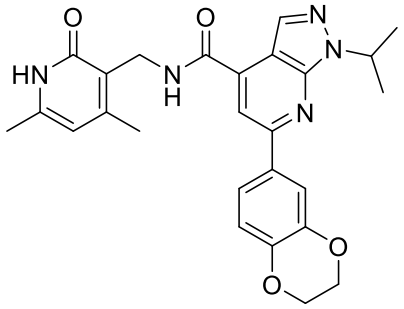
HTS

Initial HTS of 175,000 compounds against WT PRC2

Expansion screen of 5,000 compounds of related structures to hits identified in initial screen



1, initial HTS hit
IC_{50} = 3.4 ± 0.9 μM

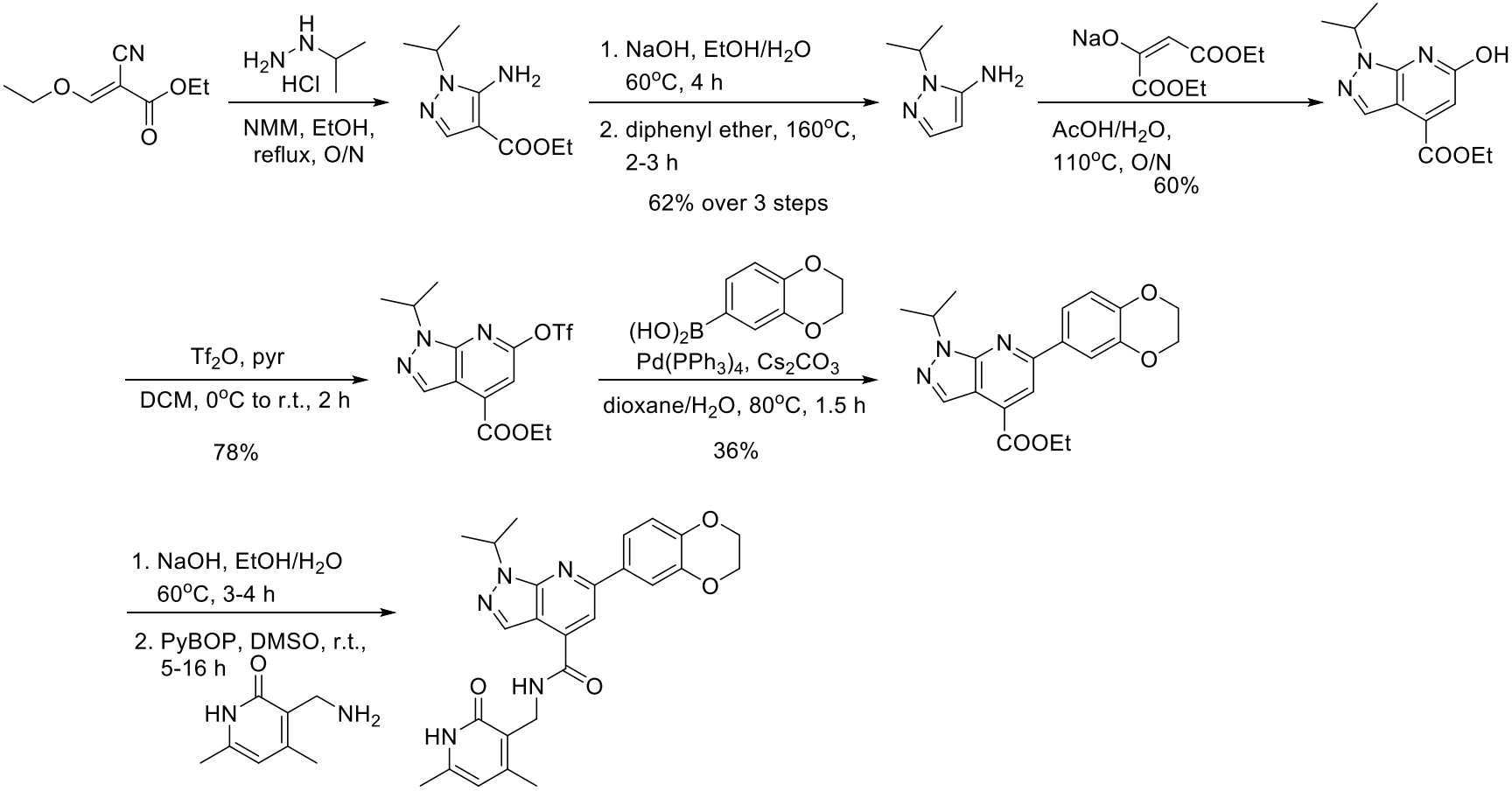


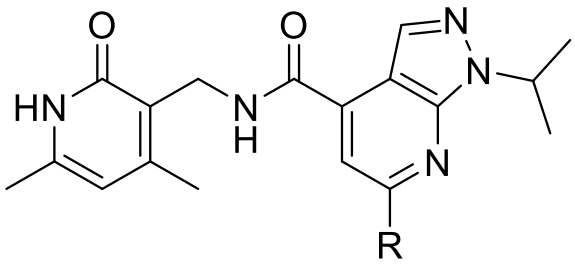
5, hit expansion
IC_{50} = 0.5 ± 0.2 μM

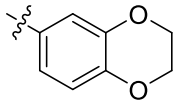
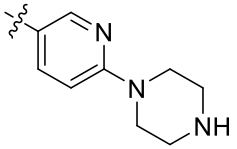
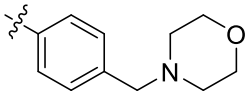
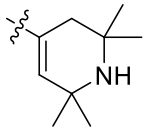
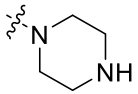
Problem: low solubility (<10 μM @ pH 7)
low oral bioavailability (0.5%)

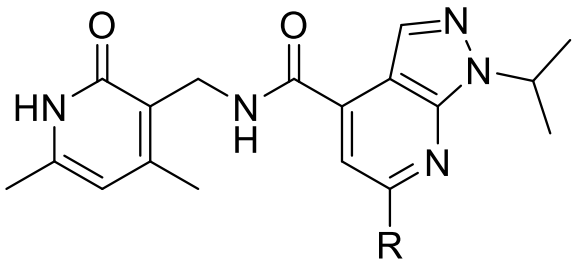
Found to be SAM competitive and nucleosome noncompetitive

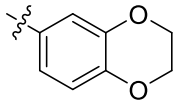
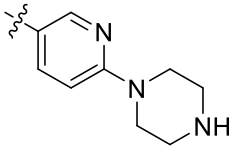
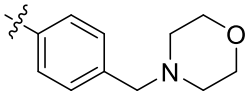
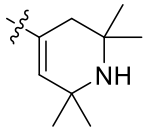
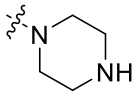
Synthesis


CN
O
OEt
O
H
N
H2N
HCl
NMM, EtOH,
reflux, O/N
NH2
N
N
COOEt
1. NaOH, EtOH/H2O
60°C, 4 h
2. diphenyl ether, 160°C,
2-3 h
62% over 3 steps
NH2
N
N
NaO
COOEt
COOEt
AcOH/H2O,
110°C, O/N
60%
OH
N
N
N
COOEt
Tf2O, pyr
DCM, 0°C to r.t., 2 h
78%
OTf
N
N
N
COOEt
(HO)2B
O
O
Pd(PPh3)4, Cs2CO3
dioxane/H2O, 80°C, 1.5 h
36%
O
O
N
N
N
COOEt
1. NaOH, EtOH/H2O
60°C, 3-4 h
2. PyBOP, DMSO, r.t.,
5-16 h
O
HN
NH2
O
N
N
N
O
O
HN
O
HN
O
HN

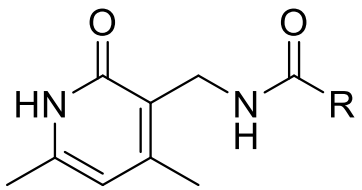


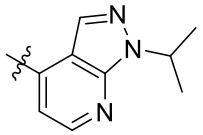
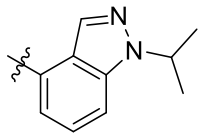
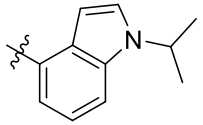
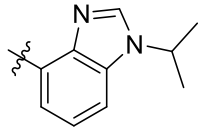
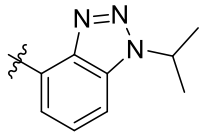
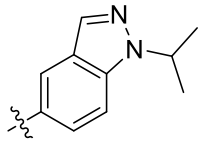
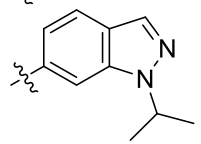
Cmpd#	R	EZH2 IC_{50} (uM)	cLogD
5		0.5 ± 0.2	2.1
6		0.2 ± 0.2	0.1
7		0.4 ± 0.2	2.1
8		0.3 ± 0.2	-0.1
9		1.6 ± 0.4	-0.7
10	iPr	2.3 ± 0.3	1.9
11	OMe	2.4 ± 0.1	0.9
12	Br	0.9 ± 0.3	1.5
13	H	3.1 ± 1.0	0.5

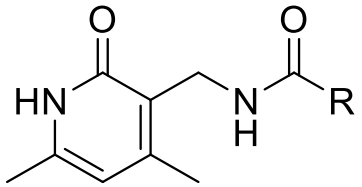


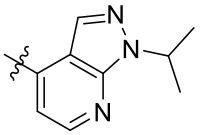
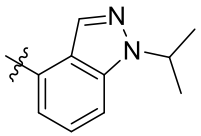
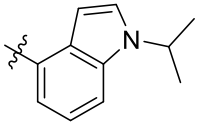
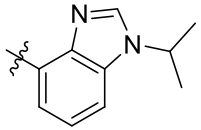
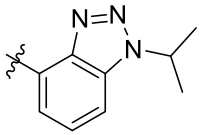
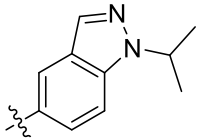
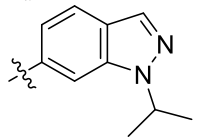
Cmpd#	R	EZH2 IC_{50} (uM)	cLogD
5		0.5 ± 0.2	2.1
6		0.2 ± 0.2	0.1
7		0.4 ± 0.2	2.1
8		0.3 ± 0.2	-0.1
9		1.6 ± 0.4	-0.7
10	iPr	2.3 ± 0.3	1.9
11	OMe	2.4 ± 0.1	0.9
12	Br	0.9 ± 0.3	1.5
13	H	3.1 ± 1.0	0.5

F = 41%

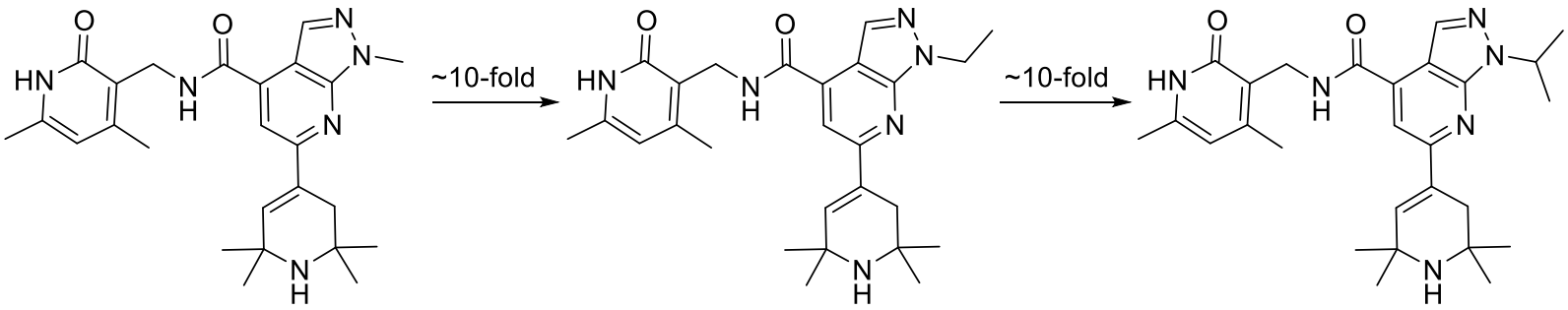


Cmpd#	R	EZH2 IC_{50} (uM)	cLogD
13		3.1 ± 1.0	0.5
14		1.1 ± 0.3	1.4
15		3.1 ± 2.6	2.2
16		>50	1.4
17		>50	1.4
18		22 ± 6	1.4
19		>50	1.4



Cmpd#	R	EZH2 IC_{50} (uM)	cLogD
13		3.1 ± 1.0	0.5
14		1.1 ± 0.3	1.4
15		3.1 ± 2.6	2.2
16		>50	1.4
17		>50	1.4
18		22 ± 6	1.4
19		>50	1.4

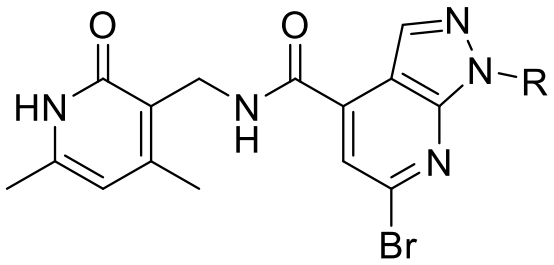
N-Alkylation

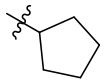
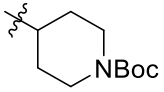
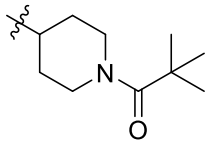
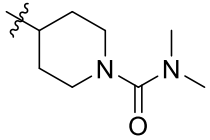
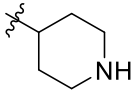
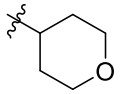


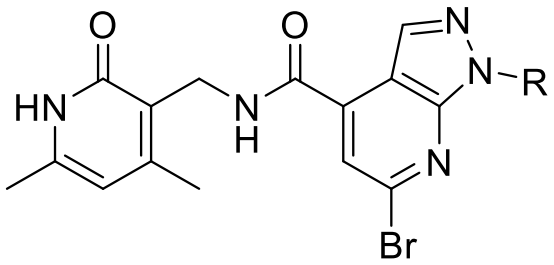
20
39 ± 13 μM

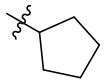
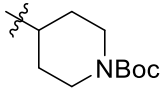
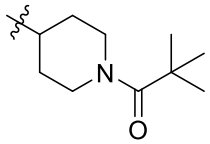
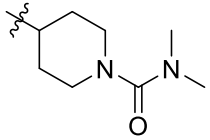
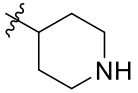
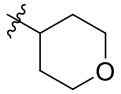
21
5 ± 3 μM

8
0.3 ± 0.2 μM



Cmpd#	R	EZH2 IC_{50} (uM)	cLogD
22		0.08 ± 0.04	2.7
23		0.2 ± 0.03	2.3
24		0.05 ± 0.03	2.4
25		0.05 ± 0.02	0.7
26		2.2 ± 1.1	-1.6
27		0.7 ± 0.2	1.3

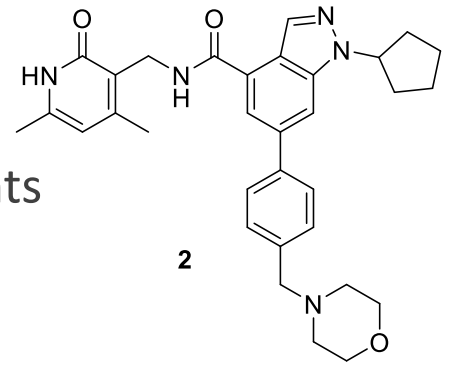


Cmpd#	R	EZH2 IC_{50} (uM)	cLogD
22		0.08 ± 0.04	2.7
23		0.2 ± 0.03	2.3
24		0.05 ± 0.03	2.4
25		0.05 ± 0.02	0.7
26		2.2 ± 1.1	-1.6
27		0.7 ± 0.2	1.3

PK

Cmpd#	ELISA EC_{50} (uM)	Cl (mL/min/kg)	AUC (h·ng/mL)	%F
5[a]	ND	24	700	0.5
7	10.5 ± 2	35	950	41
2[b]	2.9 ± 1	11	2900	47

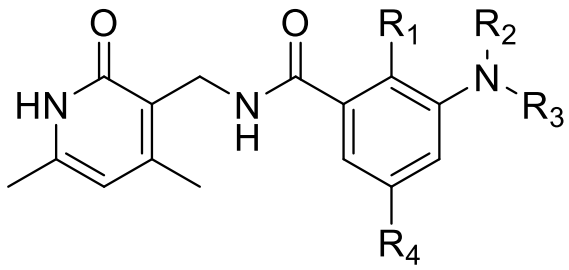
[a] 1 mg/kg IV; [b] cLogD = 3.1



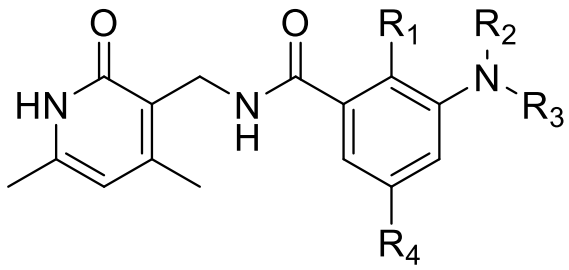
2

Many additional analogs made, only modest improvements in activity made

- When activity improved, bioavailability ↓ or clearance ↑

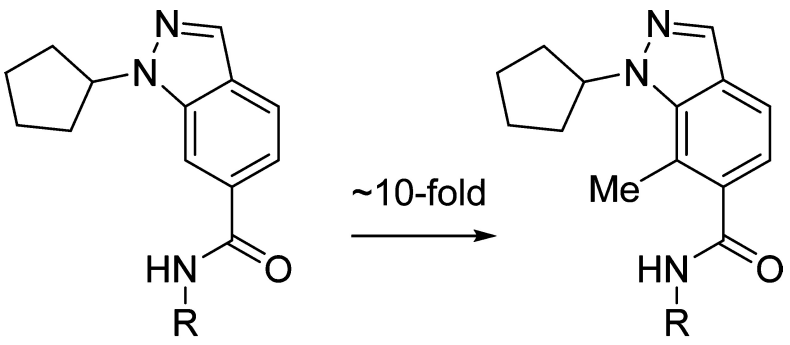


Cmpd#	R_1	R_2	R_3	R_4	EZH2 IC_{50} (uM)	cLogD
28	H	Me	*c*-Pentyl	Br	5.7 ± 1.6	3.4
29	Me	Me	*c*-Pentyl	Br	0.03 ± 0.02	3.9
30	Me	H	4-THP	Br	0.5 ± 0.2	1.9
31	Me	Me	4-THP	Br	0.03 ± 0.02	2.5
32	Me	Me	H	Cl	2.7 ± 1.2	1.7
33	Me	Me	Me	Cl	1.7 ± 0.9	2.4
34	Me	Et	4-THP	Cl	0.015 ± 0.01	2.7
35	H	Et	4-THP	Cl	14 ± 2	2.2
36	Me	Et	4-THP	Br	0.01 ± 0.01	2.9
37	Me	Me	4-Piperidine	Br	0.03 ± 0.02	-0.3



Cmpd#	R_1	R_2	R_3	R_4	EZH2 IC_{50} (uM)	cLogD	
28	H	Me	*c*-Pentyl	Br	5.7 ± 1.6	3.4	
29	Me	Me	*c*-Pentyl	Br	0.03 ± 0.02	3.9	
30	Me	H	4-THP	Br	0.5 ± 0.2	1.9	
31	Me	Me	4-THP	Br	0.03 ± 0.02	2.5	
32	Me	Me	H	Cl	2.7 ± 1.2	1.7	
33	Me	Me	Me	Cl	1.7 ± 0.9	2.4	
34	Me	Et	4-THP	Cl	0.015 ± 0.01	2.7	F = 1.6%
35	H	Et	4-THP	Cl	14 ± 2	2.2	
36	Me	Et	4-THP	Br	0.01 ± 0.01	2.9	
37	Me	Me	4-Piperidine	Br	0.03 ± 0.02	-0.3	

Magic Methyl Effect



38
>50 μM

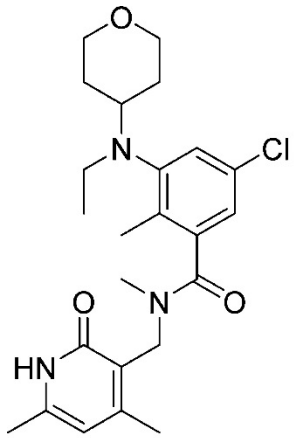
39
6 ± 2 μM

Shows that molecular shape is important for potency

o-Me forces substituents out of plane

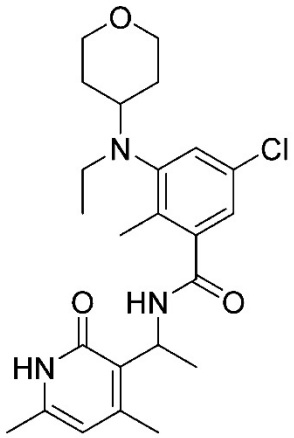
- Key for potent compounds
- Data mining found a preference for torsion angle for 2° amide adjacent to Me to between 60-140° or 220-300°
- Calculated disubstituted aniline to prefer to be out of plane from adjacent Me

Optimization of %F



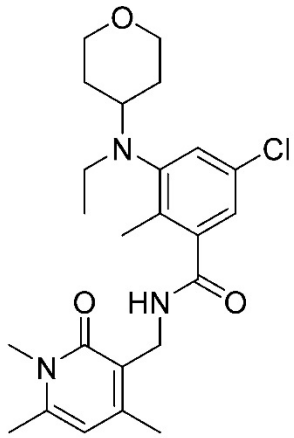
40
0.2 ± 0.1 μM

~10-fold ↓



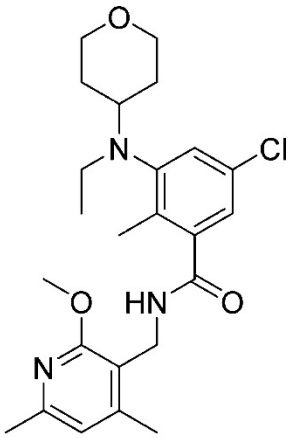
41
3 ± 1 μM

~100-fold↓



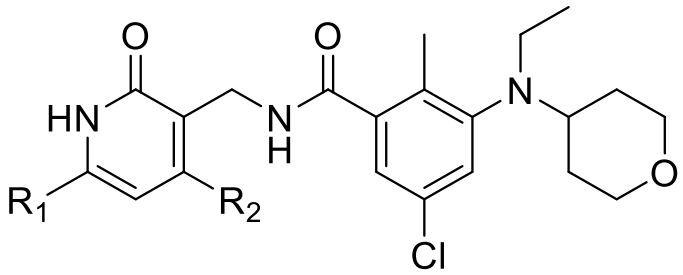
42
0.1 ± 0.1 μM

~10-fold↓

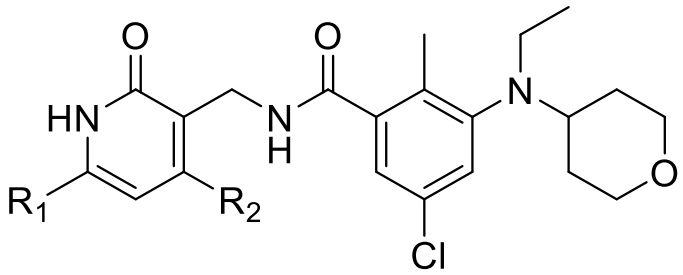


43
18 ± 8 μM

* lactam tautomer critical for potency

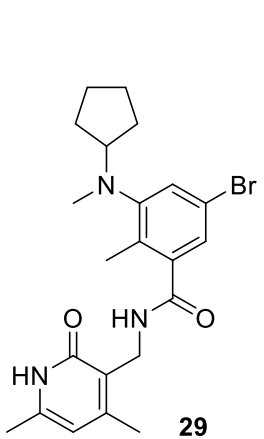


Cmpd#	R_1	R_2	EZH2 IC_{50} (uM)	cLogD
34	Me	Me	0.01 ± 0.01	2.7
44	H	H	3.3 ± 1.2	2.2.
45	H	Me	0.1 ± 0.04	2.5
46	Me	H	0.2 ± 0.01	2.4
47	Me	CH_2OH	0.2 ± 0.03	1.4
48	Me	CF_3	0.03 ± 0.01	3.0
49	Me	Et	0.01 ± 0.01	3.1
50	Me	CH_2NMe_2	0.9 ± 0.4	0.9
51	Et	Me	0.02 ± 0.01	3.2



Cmpd#	R_1	R_2	EZH2 IC_{50} (uM)	cLogD
34	Me	Me	0.01 ± 0.01	2.7
44	H	H	3.3 ± 1.2	2.2.
45	H	Me	0.1 ± 0.04	2.5
46	Me	H	0.2 ± 0.01	2.4
47	Me	CH_2OH	0.2 ± 0.03	1.4
48	Me	CF_3	0.03 ± 0.01	3.0
49	Me	Et	0.01 ± 0.01	3.1
50	Me	CH_2NMe_2	0.9 ± 0.4	0.9
51	Et	Me	0.02 ± 0.01	3.2

Key Compounds



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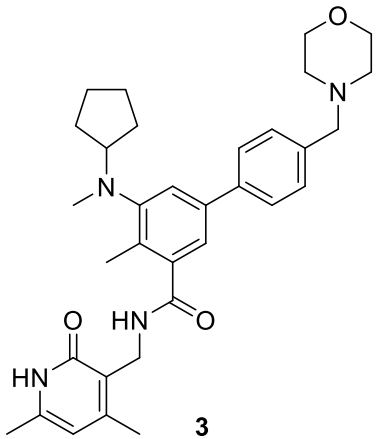

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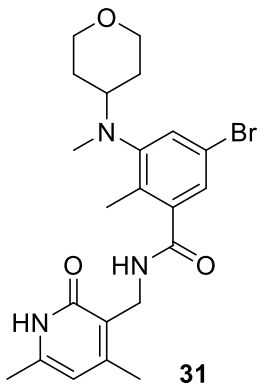

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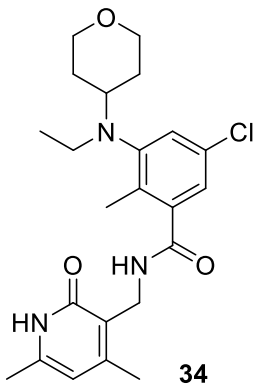

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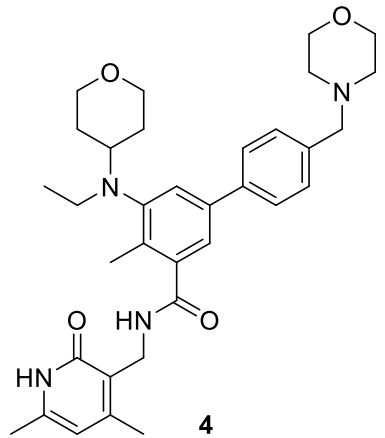
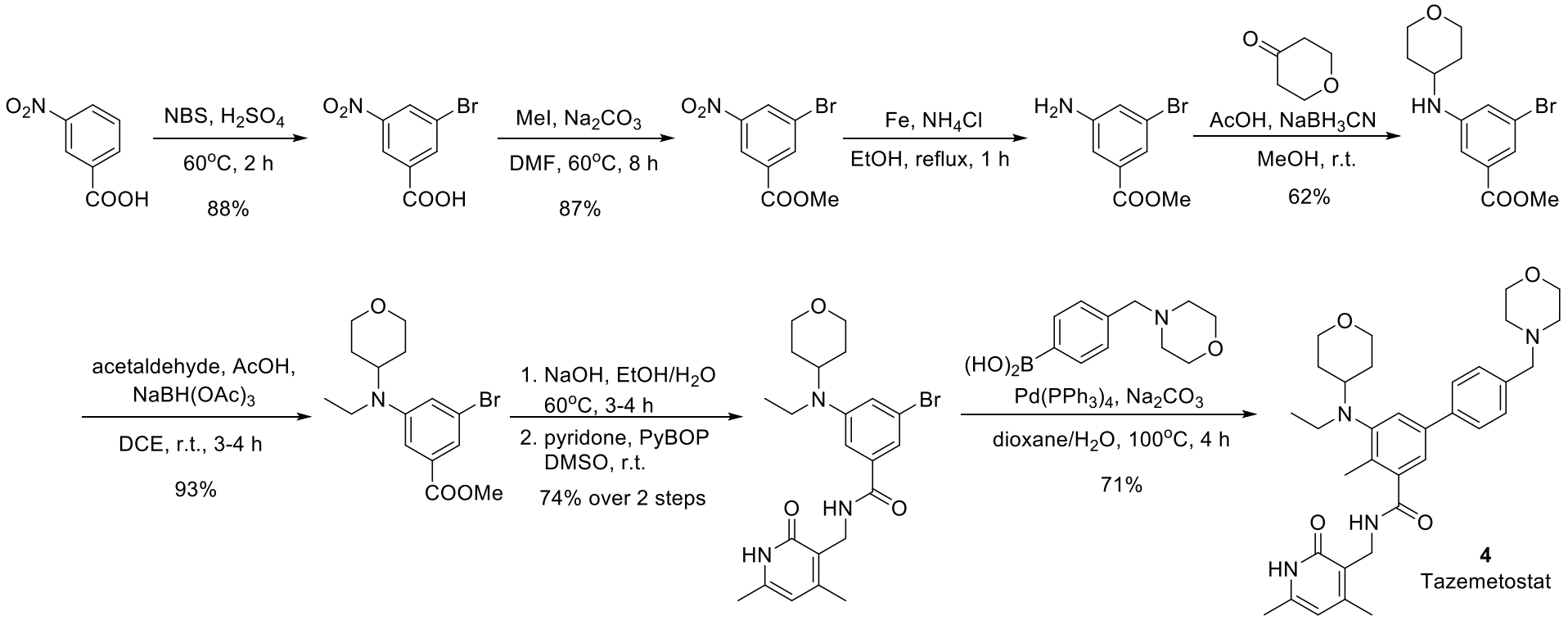


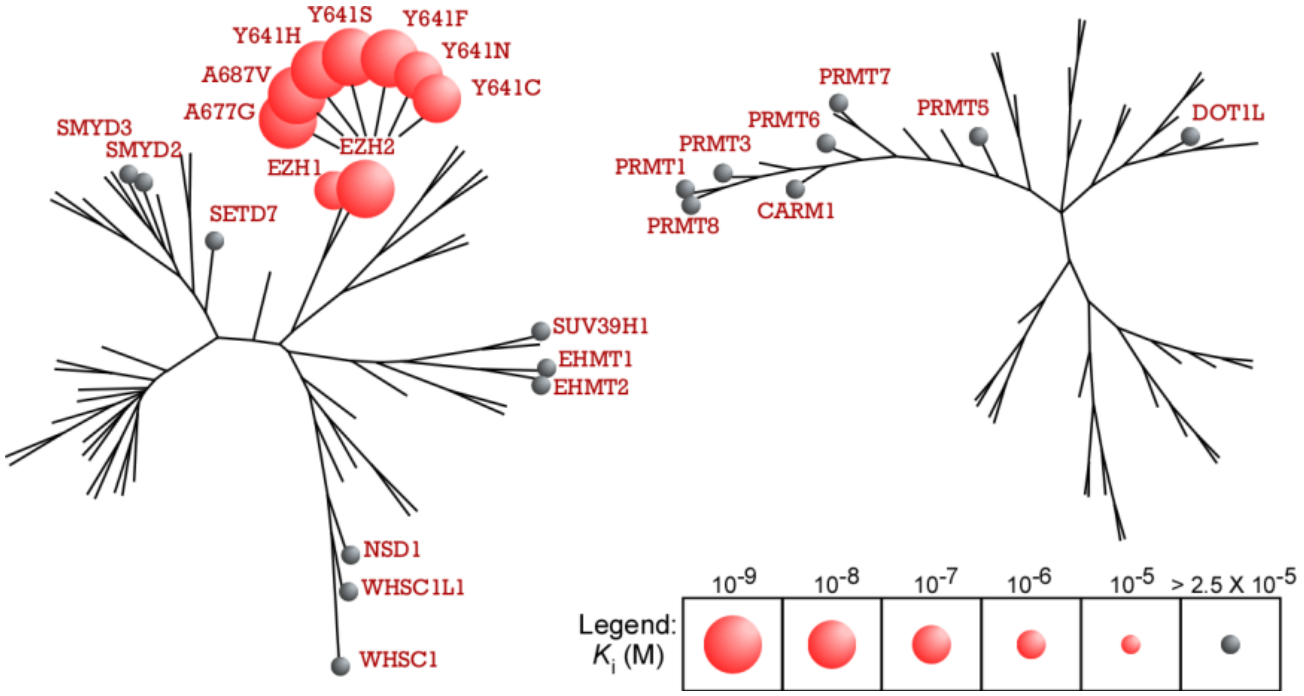
4

Cmpd#	ELISA EC_{50} (uM)	Cl (mL/min/ kg)	AUC (h·ng/ mL)	%F	cLogD
29	0.5	18	46	2	3.9
3	0.7 ± 0.2	16	2500	24	4.2
31	0.3 ± 0.1	90	350	18	2.5
34	0.3 ± 0.1	49	680	1.6	2.7
4	0.2 ± 0.1	13	2500	55	3.2

Synthesis of **4**/ Tazemetostat

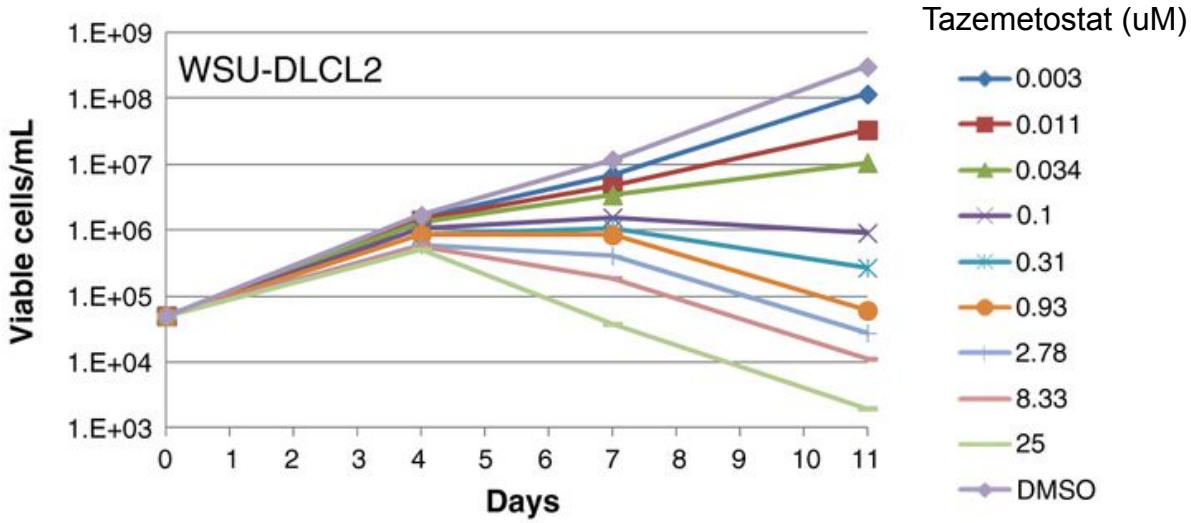


Selectivity



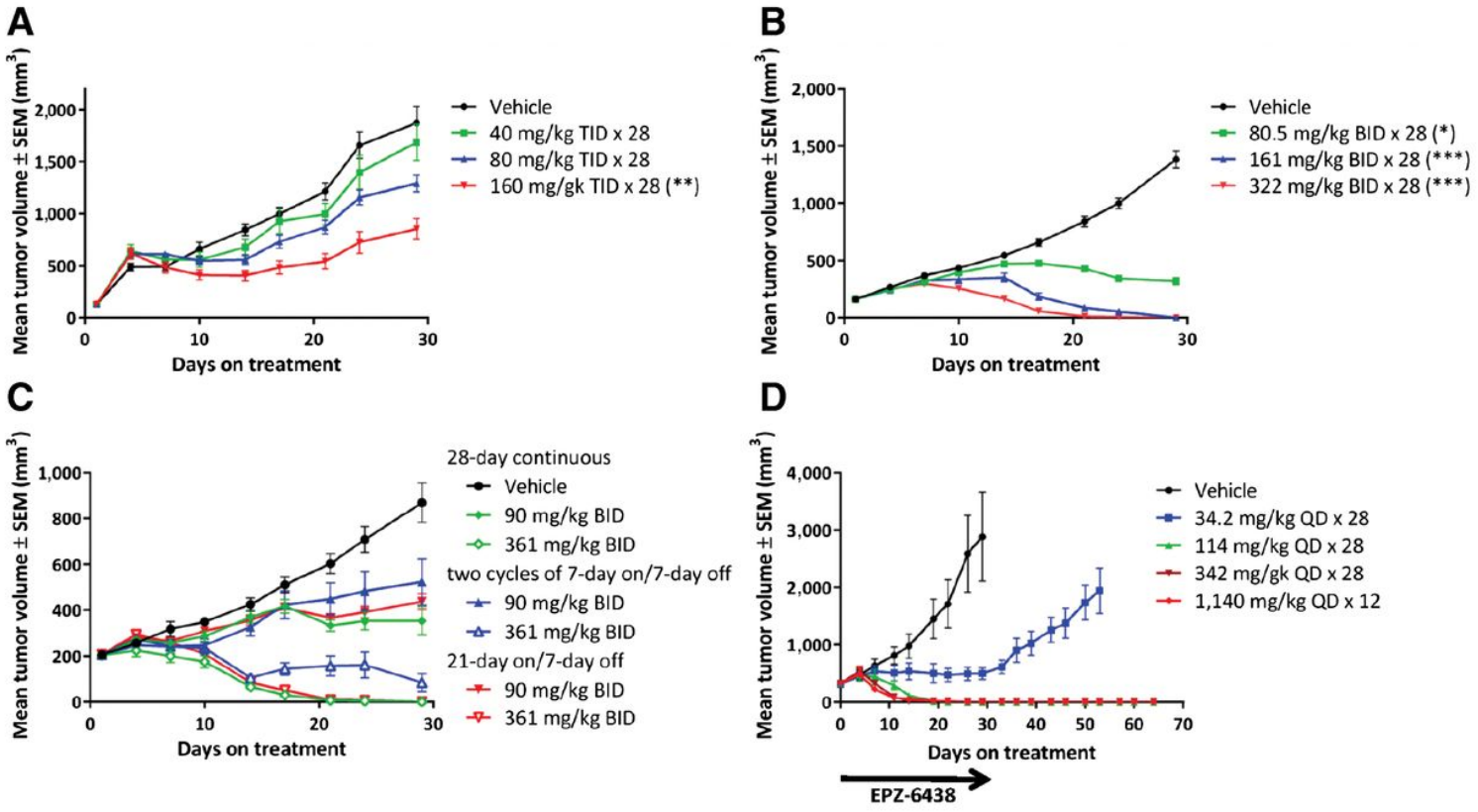
Ribrag, V. et al. Phase 1 Study of EPZ-6438 (E7438), an Enhancer of Zeste Homolog-2 (EZH2) Inhibitor: Dose Determination and Preliminary Activity in Non-Hodgkin Lymphoma. Presented at 13th International Conference on Malignant Lymphoma, Lugano, Switzerland 2015

In vitro Efficacy



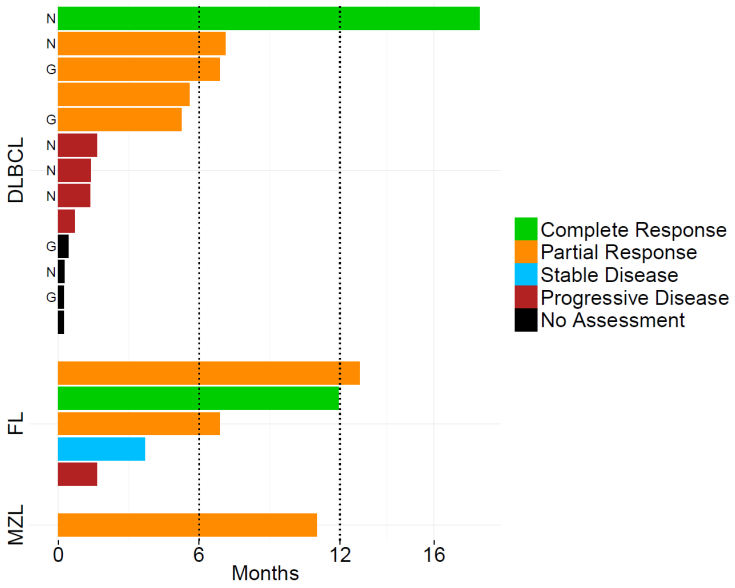
Mol. Cancer Ther. **2014**, *13*, 842-854

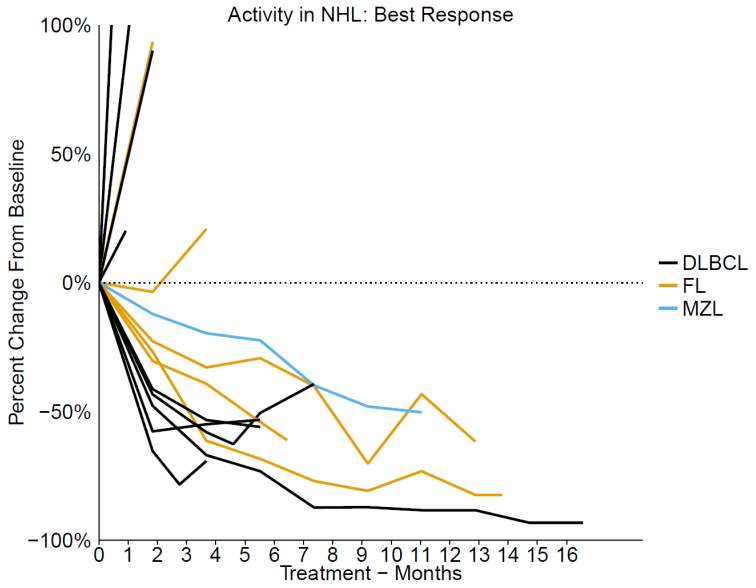
In vivo Efficacy



Mol. Cancer Ther. **2014**, *13*, 842-854

Phase I





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Summary

Optimized an EZH2 inhibitor from initial HTS screen with modest activity and poor properties through lead (good activity, improved properties) to clinical candidate which shows efficacy in patients with genetically defined tumors

In replacing a fused heterocycle with a methyl substituted aniline the power of the magic methyl was seen, at least 25-fold improvement

Magic methyl's found to be present throughout tazemetostat

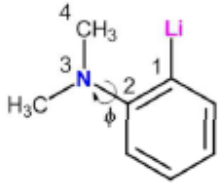
Progressing through clinical trials

- 800 mg BID (twice daily) dose to be used as recommended Phase 2 dose
- Responses seen in patients diagnosed with wild-type and mutant EZH2 tumors
- Currently recruiting pediatric patients for new Phase I trial
- Currently recruiting patients for new Phase II trial
- IND approved for additional Phase II trial

2° Amide Preferred Torsion Angles (Data Mining)

Fig. S2. Spotfire™ plot of available structural data from the Cambridge Structural Database (CSD, pink) and the Protein Date Bank (PDB, blue) showing torsion angle preference for a secondary amide with an ortho Li = Me (top) or Li = H (bottom). Analysis suggests that ortho methyl substitution favors the range of amide torsion angle from 60-140° or 220-300°. Based on the SAR and this analysis we predict that the preferred binding conformation will be within this range.

Preferred Torsion Angle of Disubstituted Aniline (Calculated)



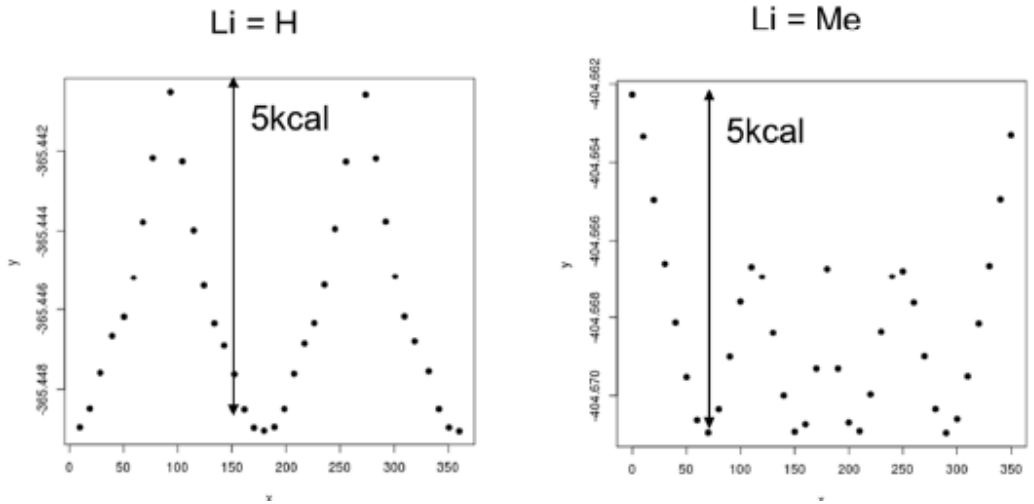


Fig. S3. Energy calculations for the preferred torsion angle (ϕ) of a dimethyl substituted aniline when Li = H, or Me. Analysis shows an orientation of 180° (in plane) is heavily favored for Li = H, whereas there exists a greater preference for the aplanar amide when Li = Me with an energy minimum at 30° out of plane (180°) and at 70° and 290°. Structural data from the Cambridge Structural Database (CSD, pink) and the Protein Date Bank (PDB, blue) was insufficient to infer binding preference for a disubstituted aniline with adjacent methyl substitution.