

Discovery and Optimization of Tetramethylpiperidinyl Benzamides as Inhibitors of EZH2

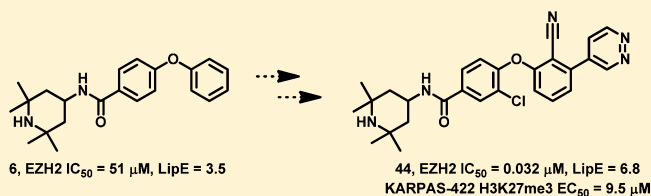
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S Supporting Information

ABSTRACT: The identification and development of a novel series of small molecule Enhancer of Zeste Homologue 2 (EZH2) inhibitors is described. A concise and modular synthesis enabled the rapid development of structure–activity relationships, which led to the identification of **44** as a potent, SAM-competitive inhibitor of EZH2 that dose-dependently decreased global H3K27me3 in KARPAS-422 lymphoma cells.

KEYWORDS: methyltransferase, PRC2, EZH2, KARPAS-422, tetramethylpiperidine, diphenylether



Histone lysine methyltransferases (HKMTs; HMTs when referred to both lysine and arginine methyltransferases) contribute to the organization of chromatin structure, and thus play a role in the regulation of gene expression. Enhancer of Zeste Homologue 2 (EZH2) catalyzes methylation of histone H3 lysine 27 (H3K27) and functions as part of a multisubunit complex termed Polycomb Repressive Complex 2 (PRC2) with known application in regulating cell identity.¹ PRC2/EZH2 has been widely implicated in cancer progression, largely due to its prevalent overexpression, which correlates with significant increases in H3K27 methylation, disease stage, and poor prognosis.^{2,3} The identification of recurrent gain of function mutations within the EZH2 catalytic domain provides a powerful argument that certain cancers might be dependent on EZH2 catalytic activity. All identified mutated residues, Y641, A677, and A687, alter substrate specificity, facilitating the conversion from H3K27 dimethylated (me2) to trimethylated (me3) states,^{4–8} while wild-type (wt) EZH2 preferentially converts H3K27me1 to H3K27me2. To date, EZH2 gain of function mutations have been found exclusively in germinal center B-cell like diffuse large B-cell lymphoma (GCB-DLBCL) and Follicular Lymphoma (FL) but not in activated B-cell DLBCL (ABC-DLBCL) or any other lymphoma subtype, suggesting that EZH2 addiction might be restricted to certain lymphoma subtypes.

Multiple small molecule inhibitors of EZH2 catalytic activity, bearing a common pyridone-amide motif, have recently been disclosed (Figure 1).^{9–16} It is of interest to note that the similarities between these compounds is not a result of deliberate differentiation strategies for generating novel inhibitors but rather an indication of the relative paucity of commercial and corporate compound collections for chemical matter with the appropriate structural features to inhibit this enzyme. Several pyridone-amides have been used to unveil new biology linked to the catalytic activity of EZH2. On target *in*

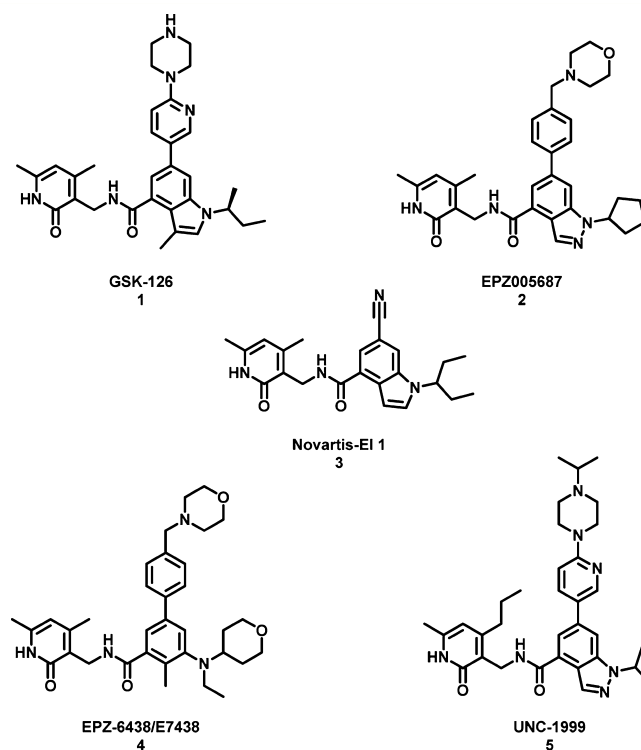


Figure 1. Disclosed EZH2 inhibitors.

vitro and *in vivo* efficacy has been demonstrated with GSK-126, EPZ-6438, and UNC-1999; where EPZ-6438 has recently progressed into phase 1 trials for the treatment of lymphoma.

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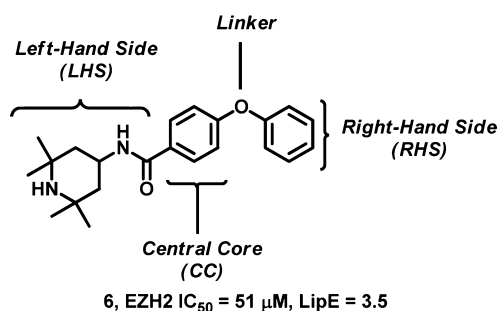


Figure 2. HTS hit compound 6.

Table 1. Linker SAR

Compound	X	EZH2wt IC ₅₀ (μM)	Compound	X	EZH2wt IC ₅₀ (μM)
6		51	13		>80
7		48	14		>80
8		>80	15		>80
9		33	16		>80
10		>80	17		>80
11		>80	18		>80
12		57			

Since all genuine inhibitors of the catalytic activity of EZH2 described to date belong to one chemical series, the pyridone-amides, the identification of structurally unrelated inhibitors enhances the chemical biology toolbox for further exploration of the function of this enzyme. Herein we describe our initial efforts in this area utilizing high throughput screening (HTS), biochemical hit triage, and hit optimization, which led to the identification of structurally distinct EZH2 inhibitors capable of suppressing EZH2 catalytic activity and globally reducing H3K27me3 in a cellular setting.

Our HTS screen utilized a PRC2 enzymatic assay that monitored ³H labeled methyl group transfer from [³H]-S-adenosyl methionine (SAM) to oligonucleosome substrate and identified 288 hits.¹⁷ These compounds were initially assessed using a multistage process: hits bearing undesirable functional groups, poor synthetic starting points, and promiscuous binders were removed. The remaining hits were assessed in 10 point concentration–response curves where the Hill coefficient (0.7–1.5) was a prominent filter for further evaluation.^{18,19}

Compounds were then assessed in three additional enzymatic assays where titrations were run with (1) 10-fold enzyme, (2) 10-fold *K_m* SAM, or (3) 10-fold *K_m* oligonucleosome substrate. These assays allowed another check for nonideal behavior and a preliminary categorization of compounds based on potential mechanism of inhibition (MOI). This MOI assessment was based on whether they

Table 2. Right-Hand Aryl Ring SAR

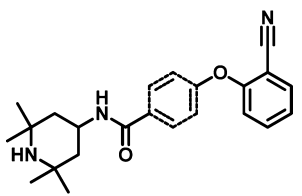
Compound	Right-Hand Side	EZH2wt IC ₅₀ (μM)	LipE
19		48	3.0
20		61	2.9
21		>80	N/A
22		29	3.2
23		59	3.6
24		3.6	3.1
25		7.9	2.8
26		19	3.8
27		3.4	4.8

did or did not shift in potency at cofactor and substrate concentrations of 10-fold *K_m*. According to the Cheng–Prusoff relationship, an increase in substrate above *K_m* should result in predictable decreased inhibitor affinity for a competitive compound, no effect for a noncompetitive compound, and enhanced affinity for an uncompetitive one.^{17,20}

After hit triage was complete, several relatively weak (IC₅₀ > 20 μM) compounds remained, which met the criteria outlined above. One of these, compound 6 (Figure 2), was of moderate potency (EZH2_{wt} IC₅₀ = 51 μM), had a good Hill coefficient (1.1), and LipE of 3.5.^{21,22} When funneled through our secondary assays 6 shifted only with an increase in [SAM], which was suggestive of a SAM competitive mechanism of inhibition.¹⁷ Additionally, 6 was found to be selective when assessed against other HMTs in a biochemical assay.¹⁷ Given these data and the potential for rapid synthesis of analogues, 6 was selected as a starting point for our initial efforts to develop wild-type and disease-relevant mutant EZH2 inhibitors.

The aim of our initial medicinal chemistry campaign was to rapidly navigate the structure–activity landscape of tetraethylpiperidinyll-containing lead compound 6 (Table 1). We first addressed the linker of the bis-phenylether by extending the compound one methylene unit (–CH₂O–, 7), which

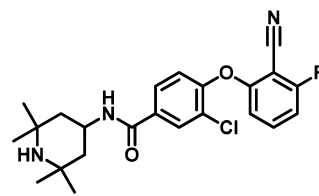
Table 3. Central Core SAR



Compound	Central Core	EZH2wt IC ₅₀ (μM)	LipE
28		15	4.0
29		37	3.2
30		>80	N/A
31		0.84	4.4
32		1.0	5.2
33		0.92	4.9
34		0.93	5.5
35		0.50	4.9
36		0.45	5.1
37		0.32	5.1
38		1.0	4.4
39		51	2.6

maintained activity. The converse methylene insertion (–OCH₂–, **8**) led to a loss of EZH2 affinity. Conversion of ether **6** to thioether **9** maintained potency; however, oxidation of the sulfur led to ablation of activity (Table 1, **10** and **11**). Interestingly, ketone **12** was tolerated, while the corresponding racemic alcohol **13** was not. At this point, we postulated that the ether, thio, and ketone linkages were imparting a conformational bias to the system, which led to enhanced potency over the sulfoxide, sulfone, and benzylic alcohol. Indeed, diphenyl ethers are known to adopt a twisted orientation due to a 1,5-CH-aryl clash and optimized π -overlap with the lone pairs on the ether oxygen.^{23,24} Diphenylamine is known to adopt a similar conformation to benzophenone, which is more coplanar than the conformation of diphenyl

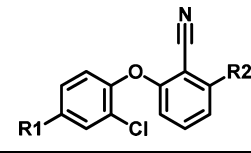
Table 4. Further Modification of Right-Hand Aryl Ring



Compound	R	EZH2wt IC ₅₀ (μM)	EZH2 IC ₅₀ (μM) ^a	LipE(wt)
40		0.14	1.4	5.1
41		0.14	2.2	5.0
42		0.23	4.0	5.4
43		0.081	0.56	5.4
44		0.032	0.19	6.8
45		0.024	0.13	6.3

^aY641N mutant EZH2 used.

Table 5. Tetramethylpiperidine SAR

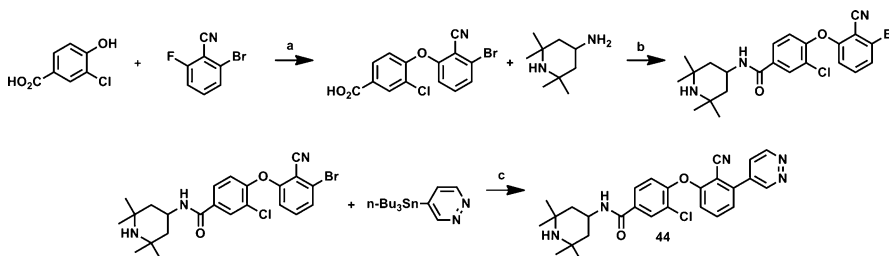


Compound	R1	R2	EZH2wt IC ₅₀ (μM)	EZH2 IC ₅₀ (μM) ^a	A to B	B to A	Efflux Ratio ^b
44			0.032	0.19	0.19	3.29	17
46			4.9	62	N/A	N/A	N/A
47			>80	>80	N/A	N/A	N/A
48			1.5	66	21.9	24.8	1.1
49			0.871	10	N/A	N/A	N/A
50			2.1	10	N/A	N/A	N/A
51			0.172	2.5	20.4	47.7	2.3
52			0.189	2.0	0.5	2.1	4.4

^aY641N mutant EZH2 used. ^bCACO-2 permeability assay.

ethers;^{24,25} therefore, absence of activity for compounds **13** and **14** could be attributed to preference of EZH2 for H-bond acceptors and not donors as a linking group in addition to an optimum conformation between the phenyl rings. This hypothesis was further supported by the lack of activity for

Scheme 1. Synthesis of Compound 44



(a) K_2CO_3 , DMSO, 140 °C, 3 h, 81% yield; (b) EDC, HOBT, TEA, DCM, 23 °C, 3 h, 30% yield; (c) 10 mol% $Pd(PPh_3)_2Cl_2$, LiCl, toluene, 90 °C, 12 h, 60% yield.

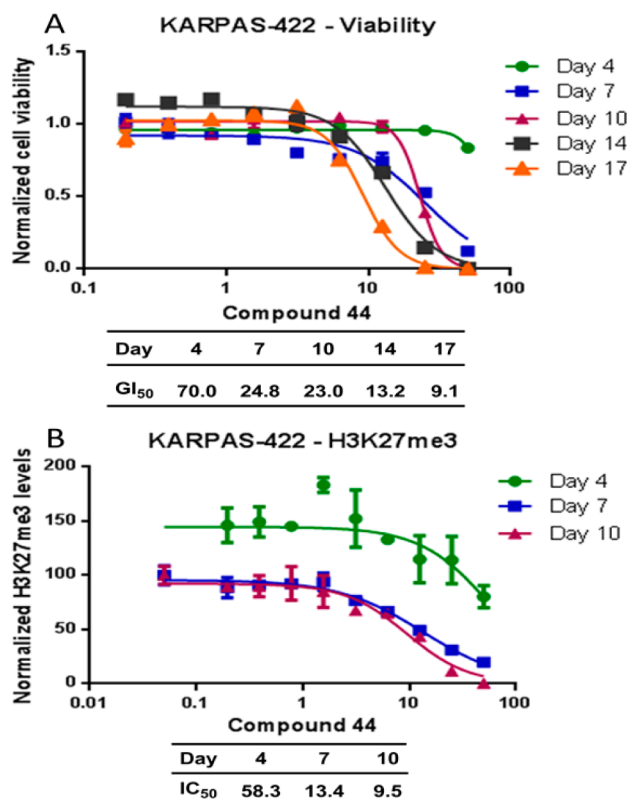


Figure 3. Activity of compound 44 in KARPAS-422 cells. (A) Viability of KARPAS-422 cells with growth inhibition observed with varying [44] and days of exposure. (B) Compound 44 treatment results in dose- and time-dependent reduction in global H3K27me3 levels in KARPAS-422 cells. Total H3 levels were determined and used for normalization purposes. Experiments were carried out in triplicate \pm SD.

carbon linked compounds 16–18, where the phenyl rings should adopt a near orthogonal orientation.

Holding the diphenylether constant, we next evaluated the right-hand aryl ring. Incorporation of a 2-methyl group (Table 2, 19) was nearly equipotent with 6, and the continued scan led to progressive loss of potency at the 3- and 4-positions (Table 2, 20 and 21). Focusing on the 2-aryl position, 2-chloro (21) provided a modest improvement, while 2-methoxy (23) was equipotent to 6. Extension of the 2-methoxy to 2-benzyloxy (24) or branching of the 2-methyl to 2-*t*-butyl (25) led to a 10-fold boost in potency with no increase in LipE, illustrating that this position was tolerant of diverse functionality and potentially reinforced an optimal conformation. A modest boost in LipE was coupled with smaller rigid groups at the 2-

position (26, LipE = 3.8). Further exploration revealed a 15-fold boost in potency with the incorporation of a 2-cyano substituent (27, LipE = 4.8), suggesting the cyano nitrogen was making a favorable interaction with the binding site in EZH2. The 2-cyano substituent was utilized as the preferred substituent for further SAR development.

We next addressed elaboration of the central aromatic ring. While substitution proximal to the amide linkage did not yield an improvement in biochemical potency relative to 27 (Table 3, 28–30), modification of the central core to a naphthyl system did provide a 4-fold enhancement (Table 3, 31). We suspected that monosubstitution adjacent to the ether linkage would stabilize the intrinsic twist of the biphenylether and thus enhance potency. We were gratified to find that this was the case as fluoro, methyl, and cyano substitution all led to a minimum of a 3-fold improvement (Table 3, 32–34). Both cyclopropyl (35) and chloro (36) substitution improved potency 6-fold over 27 with 36 being the more efficient modification (0.45 μ M, LipE = 5.1) at this position and provided a compound with meaningful levels of potency against EZH2 Y641N mutant (36, 7.9 μ M). Switching from chloro (36) to bromo (37) increased lipophilicity leading to a modest boost in potency with no increase in efficiency as indicated by the static LipE (5.1). Disubstitution of the central core did not provide an advantage (Table 3, 38 and 39). The chloro group was selected for further optimization.

In an attempt to improve the biochemical potency of the tetramethylpiperidinyl-class of inhibitors, we chose to further evaluate the right-hand aryl ring (Table 4). Methyl and chloro substitution alpha to the 2-cyano group improved binding to EZH2 wild-type and Y641N mutant 3- and 4-fold over 36, respectively (Table 4, 40 and 41). Introduction of a pyrazole alpha to the 2-cyano group (42) led to a modest improvement; however, inclusion of a 6-membered heterocycle enabled sub-100 nM potency against the wild-type enzyme (Table 4, 43–45). Changing the nitrogen aryl substitution from 4-pyridyl (43, LipE = 5.4) to pyridazine (44, LipE = 6.8) then pyrimidine (45, LipE = 6.3) led to improvement in both affinity and efficiency.

Compound 44 was evaluated in a CACO-2 assay and found to have low passive permeability and a high efflux ratio, indicative of a compound with the potential for poor oral bioavailability.^{26,27} We therefore sought to address this liability through modification of the tetramethylpiperidine (Table 5). One approach was to attenuate the basicity the nitrogen via inductive effects; unfortunately, *N*-ethanol 46 lost 10-fold against EZH2 wild-type, while difluoropiperidine 47 was inactive (compare to 36). Indeed, a strongly basic nitrogen is preferred for activity as pyran 48 was 38-fold less potent than

44. It is worthy of note that **48** no longer displays efflux (efflux ratio = 1.1) and was quite permeable (A to B 21.9×10^{-6} cm/s), lending support to the hypothesis that poor permeability and efflux were a direct result of the basic nitrogen of **44**. Further manipulation of the tetramethylpiperidine through selective methyl group deletion also provided compounds with a significant reduction in activity (**49–50**). In the face of an apparent SAR cliff around the modification of the tetramethylpiperidine, we attempted to improve the permeability of this series by manipulating the amide linker between the tetramethylpiperidine and central core. Both ester (**51**) and ether (**52**) were tolerated displaying a 4-fold loss in potency, while improving permeability and reducing efflux. The improved permeability achieved with **52** did not translate into an improved shift between biochemical potency and reduction of H3K27me3 in HeLa cells (HeLa cell assay **44** = 7 μ M;¹⁷ **52** \geq 15 μ M); therefore, compound **44** was prioritized for further evaluation as a tool compound for the study of EZH2 disease biology.

The synthesis of **44** commenced with a S_NAr reaction to establish the diphenyl ether linkage (Scheme 1). EDC-mediated peptide coupling furnished the tetramethylpiperidinyl-benzamide scaffold primed for exploring diversity on the right-hand side aryl ring. The pyridazine was coupled using standard Stille conditions to provide **44** in three chemical steps and 15% overall yield.

Time and concentration-dependent exposure of KARPAS-422 cells to **44** led to growth arrest (Figure 3A) as well as dose-dependent reduction in global H3K27me3 levels (Figure 3B). These results are consistent with previous reports and validate this scaffold as a useful tool compound to interrogate the inhibition of EZH2.^{9–16}

This work described the identification and optimization of a new class of inhibitors of the catalytic activity of EZH2/PRC2, the tetramethylpiperidinyl benzamides. Compound **6** was initially identified as a SAM-competitive compound with acceptable LipE and a modular synthesis well suited for rapid SAR development. Optimization of **6** provided **44**, a SAM competitive compound that was >1000-fold more potent against EZH2. We also showed that upon treatment with **44** KARPAS-422 lymphoma cells displayed dose-dependent reduction in global levels of H3K27me3 with a characteristic delay in onset of phenotype. Compound **44** is an effective tool for the evaluation of EZH2 inhibition *in vitro*.

■ ASSOCIATED CONTENT

📄 Supporting Information

Synthetic procedures, characterization data, the synthesis of compound **44**, and KARPAS-422 assay information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

K₂CO₃, potassium carbonate; DMSO, dimethylsulfoxide; EDC, *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride; HOBT, 1-hydroxybenzotriazole hydrate; TEA, triethylamine; DCM, dichloromethane; Pd(PPh₃)₂Cl₂, bis-(triphenylphosphine)palladium(II) dichloride; LiCl, lithium chloride; EZH2, enhancer of zeste homologue 2; PRC2, polycomb repressive complex 2; H3K27me3, histone 3 lysine 27 trimethyl

■ REFERENCES

- (1) Margueron, R.; Reinberg, D. The Polycomb Complex PRC2 and Its Mark in Life. *Nature* **2011**, *469*, 343–349.
- (2) Kleer, C. G.; Cao, Q.; Varambally, S.; Shen, R.; Ota, I.; Tomlins, S. A.; Ghosh, D.; Sewalt, R. G.; Otte, A. P.; Hayes, D. F.; Sabel, M. S.; Livant, D.; Weiss, S. J.; Rubin, M. A.; Chinnaiyan, A. M. EZH2 is a Marker of Aggressive Breast Cancer and Promotes Neoplastic Transformation of Breast Epithelial Cells. *Proc. Natl. Acad. Sci.* **2003**, *100*, 11606–11611.
- (3) Varambally, S.; Dhanasekaran, S. M.; Zhou, M.; Barrette, T. R.; Kumar-Sinha, C.; Sanda, M. G.; Ghosh, D.; Pienta, K. J.; Sewalt, R. G.; Otte, A. P.; Rubin, M. A.; Chinnaiyan, A. M. The Polycomb Group Protein EZH2 is Involved in Progression of Prostate Cancer. *Nature* **2002**, *419*, 624–629.
- (4) Majer, C. R.; Jin, L.; Scott, M. P.; Knutson, S. K.; Kuntz, K. W.; Keilhack, H.; Smith, J. J.; Moyer, M. P.; Richon, V. M.; Copeland, R. A.; Wigle, T. J. A687V EZH2 Is a Gain-of-Function Mutation Found in Lymphoma Patients. *FEBS Lett.* **2012**, *586*, 3448–3451.
- (5) McCabe, M. T.; Graves, A. P.; Ganji, G.; Diaz, E.; Halsey, W. S.; Jiang, Y.; Smitheman, K. N.; Ott, H. M.; Pappalardi, M. B.; Allen, K. E.; Stephanie, B. C.; Della Pietra, A., III; Dul, E.; Hughes, A. M.; Gilbert, S. A.; Thrall, S. A.; Tummino, P. J. Mutation of A677 in Histone Methyltransferase EZH2 in Human B-Cell Lymphoma Promotes Hypertrimethylation of Histone H3 on Lysine 27 (H3K27). *Proc. Natl. Acad. Sci.* **2012**, *109*, 2989–2994.
- (6) Sneeinger, C. J.; Scott, M. P.; Kuntz, K. W.; Knutson, S. K.; Pollock, R. M.; Richon, V. M.; Copeland, R. A. Coordinated Activities of Wild-Type Plus Mutant EZH2 Drive Tumor-Associated Hypertrimethylation of Lysine 27 on Histone H3 (H3K27) in Human B-Cell Lymphomas. *Proc. Natl. Acad. Sci.* **2010**, *107*, 20980–20985.
- (7) Wigle, T. J.; Knutson, S. K.; Jin, L.; Kuntz, K. W.; Pollock, R. M.; Richon, V. M.; Copeland, R. A.; Scott, M. P. The Y641C Mutation of EZH2 Alters Substrate Specificity for Histone H3 Lysine 27 Methylation States. *FEBS Lett.* **2011**, *585*, 3011–3014.
- (8) Yap, D. B.; Chu, J.; Berg, T.; Schapira, M.; Cheng, S. W.; Moradian, A.; Morin, R. D.; Mungall, A. J.; Meissner, B.; Boyle, M.; Marquez, V. E.; Marra, M. A.; Gascoyne, R. D.; Humphries, R. K.; Arrowsmith, C. H.; Morin, G. B.; Aparicio, S. A. J. R. Somatic Mutations at EZH2 Y641 Act Dominantly Through a Mechanism of Selectively Altered PRC2 Catalytic Activity, to Increase H3K27 Trimethylation. *Blood* **2011**, *117*, 2451–2459.
- (9) Diaz, E.; Machutta, C. A.; Chen, S.; Jiang, Y.; Nixon, C.; Hofmann, G.; Key, D.; Sweitzer, S.; Patel, M.; Wu, Z.; Creasy, C. L.; Kruger, R. G.; LaFrance, L.; Verma, S. K.; Pappalardi, M. B.; Le, B.; Van Aller, G. S.; McCabe, M. T.; Tummino, P. J.; Pope, A. J.; Thrall, S.

H.; Schwartz, B.; Brandt, M. Development and Validation of Reagents and Assays for EZH2 Peptide and Nucleosome High-Throughput Screens. *J. Biomol. Screening* **2012**, *17*, 1279–1292.

(10) Knutson, S. K.; Wigle, T. J.; Warholic, N. M.; Sneeringer, C. J.; Allain, C. J.; Klaus, C. R.; Sacks, J. D.; Raimondi, A.; Majer, C. R.; Song, J.; Scott, M. P.; Jin, L.; Smith, J. J.; Olhava, E. J.; Chesworth, R.; Moyer, M. P.; Richon, V. M.; Copeland, R. A.; Keilhack, H.; Pollock, R. M.; Kuntz, K. W. A Selective Inhibitor of EZH2 Blocks H3K27 Methylation and Kills Mutant Lymphoma Cells. *Nat. Chem. Biol.* **2012**, *8*, 890–896.

(11) McCabe, M. T.; Ott, H. M.; Ganji, G.; Korenchuk, S.; Thompson, C.; Van Aller, G. S.; Liu, Y.; Graves, A. P.; Della Pietra, A., III; Diaz, E.; LaFrance, L. V.; Mellinger, M.; Duquenne, C.; Tian, X.; Kruger, R. G.; McHugh, C. F.; Brandt, M.; Miller, W. H.; Dhanak, D.; Verma, S. K.; Tummino, P. J.; Creasy, C. L. EZH2 Inhibition as a Therapeutic Strategy for Lymphoma with EZH2-Activating Mutations. *Nature* **2012**, *492*, 108–112.

(12) Qi, W.; Chan, H.; Teng, L.; Li, L.; Chuai, S.; Zhang, R.; Zeng, J.; Li, M.; Fan, H.; Lin, Y.; Gu, J.; Ardayfio, O.; Zhang, J.-H.; Yan, X.; Fang, J.; Mi, Y.; Zhang, M.; Zhou, T.; Feng, G.; Chen, Z.; Li, G.; Yang, T.; Zhao, K.; Liu, X.; Yu, Z.; Lu, C.; Atadja, P.; Li, E. Selective Inhibition of EZH2 by a Small Molecule Inhibitor Blocks Tumor Cell Proliferation. *Proc. Natl. Acad. Sci.* **2012**, *109*, 21360–21365.

(13) Verma, S. K.; Tian, X.; LaFrance, L. V.; Duquenne, C.; Suarez, D. P.; Newlander, K. A.; Romeril, S. P.; Burgess, J. L.; Grant, S. W.; Brackley, J. A.; Graves, A. P.; Scherzer, D. A.; Shu, A.; Thompson, C.; Ott, H. M.; Van Aller, G. S.; Machutta, C. A.; Diaz, E.; Jiang, Y.; Johnson, N. W.; Knight, S. D.; Kruger, R. G.; McCabe, M. T.; Dhanak, D.; Tummino, P. J.; Creasy, C. L.; Miller, W. H. Identification of Potent, Selective, Cell-Active Inhibitors of the Histone Lysine Methyltransferase EZH2. *ACS Med. Chem. Lett.* **2012**, *3*, 1091–1096.

(14) Konze, K. D.; Ma, A.; Li, F.; Barsyte-Lovejoy, D.; Parton, T.; MacNevin, C. J.; Liu, F.; Gao, C.; Huang, X.-P.; Kuznetsova, E.; Rougie, M.; Jiang, A.; Pattenden, S. G.; Norris, J. L.; James, L. I.; Roth, B. L.; Brown, P. J.; Frye, S. V.; Arrowsmith, C. H.; Hahn, K. M.; Wang, G. G.; Vedadi, M.; Jin, J. An Orally Bioavailable Chemical Probe of the Lysine Methyltransferases EZH2 and EZH1. *ACS Chem. Biol.* **2013**, *8*, 1324–1334.

(15) Kuntz, K. W.; Chesworth, R.; Duncan, K. W.; Keilhack, H.; Warholic, N.; Klaus, C.; Kathleen, S. K.; Wigle, T. J. N.; Seki, M.; Shirotori, S.; Kawano, S. Aryl- or Heteroaryl-Substituted Benzene Compounds. U.S. Patent Appl. 20130123234, 2013.

(16) Sweis, R. F.; Michaelides, M. R. Chapter Thirteen: Recent Advances in Small-Molecule Modulation of Epigenetic Targets: Discovery and Development of Histone Methyltransferase and Bromodomain Inhibitors. *Annu. Rep. Med. Chem.* **2013**, *48*, 185–203.

(17) Shivani Garapaty-Rao, S.; Navveschuk, C. G.; Gagnon, A.; Chan, E. Y.; Sandy, P.; Busby, J.; Balasubramanian, S.; Campbell, R.; Zhao, F.; Bergeron, L.; Audia, J. E.; Albrecht, B. K.; Harmange, J.-C.; Cummings, R.; Trojer, P. Identification of EZH2 and EZH1 Small Molecule Inhibitors with Selective Impact on Diffuse Large B Cell Lymphoma Cell Growth. *Chem. Biol.* **2013**, *20*, 1329–1339.

(18) Shoichet, B. K. Interpreting Steep Dose-Response Curves in Early Inhibitor Discovery. *J. Med. Chem.* **2006**, *49*, 7274–7277.

(19) Under standard assay conditions, genuine inhibitors should have Hill coefficients of 1 while those with undesired mechanisms of inhibition, e.g., covalent modifiers, promiscuous aggregators, etc., generally have steeper Hill coefficients. We chose as a cutoff those that had Hill coefficients of 0.7–1.5 for further study.

(20) Cheng, Y.; Prusoff, W. H. Relationship Between the Inhibition Constant (K_i) and the Concentration of Inhibitor, Which Causes 50 Per Cent Inhibition (I_{50}) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

(21) Shultz, M. D. Improving the Plausibility of Success with Inefficient Metrics. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5980–5991.

(22) Shultz, M. D. The Thermodynamic Basis for the Use of Lipophilic Efficiency (LipE) in Enthalpic Optimizations. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5992–6000.

(23) Dahlgard, M.; Brewster, R. Q. Absorption Spectra of Some Highly Substituted Diaryl Ethers. *J. Am. Chem. Soc.* **1958**, *80*, 5861–5863.

(24) Gusakova, N. N.; Pankratov, A. N.; Fedorov, E. E.; Mushtakova, S. P.; Gribov, L. A. Calculation of the Conformation of Diphenylamine and its *ortho*- and *para*-Nitro-Derivatives by the Method of Atom–Atom Potentials. *J. Struct. Chem.* **1981**, *22*, 341–345.

(25) Brameld, K. A.; Kuhn, B.; Reuter, D. C.; Stahl, M. Small Molecule Conformational Preferences Derived from Crystal Structure Data. A Medicinal Chemistry Focused Analysis. *J. Chem. Inf. Model.* **2008**, *48*, 1–24.

(26) Thomas, V. H.; Bhattachar, S.; Hitchingham, L.; Zocharski, P.; Naath, M.; Surendran, N.; Stoner, C. L.; El-Kattan, A. The Road Map to Oral Bioavailability: An Industrial Perspective. *Expert Opin. Drug Metab. Toxicol.* **2006**, *2*, 591–608.

(27) For additional ADME and in vivo characterization of **44**, please see the Supporting Information.