Letter

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Identification of Potent, Selective, Cell-Active Inhibitors of the Histone Lysine Methyltransferase EZH2

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Supporting Information

ABSTRACT: The histone H3-lysine 27 (H3K27) methyltransferase EZH2 plays a critical role in regulating gene expression, and its aberrant activity is linked to the onset and progression of cancer. As part of a drug discovery program targeting EZH2, we have identified highly potent, selective, SAM-competitive, and cell-active EZH2 inhibitors, including GSK926 (**3**) and GSK343 (**6**). These compounds are small molecule chemical tools that would be useful to further explore the biology of EZH2.

KEYWORDS: *Epigenetics, EZH2, H3K27me3, methyltransferase, PRC2, SAM-competitive inhibitor*

Numerous studies suggest that aberrant, post-translational, histone modifications play an important role in the initiation and progression of several diseases.[1−4] In this context, the histone lysine methyltransferase EZH2 (Enhancer of Zeste Homologue 2), the catalytic member of the Polycomb Repressive Complex 2 (PRC2), has been implicated in the progression of cancer. EZH2 catalyzes the methylation of the ε-NH_2 group of histone 3 lysine 27 (H3K27) in the nucleosome substrate, via transfer of a methyl group from the cofactor *S*-(*S′*-adenosyl)-L-methionine (SAM), leading to trimethylation of H3K27 (H3K27me3) and transcriptional silencing of target genes.[5] Several studies show that elevated levels of EZH2 correlate with poor prognosis in solid tumors, including those in prostate, breast, kidney, and lung.[6−9] More recently, somatic activating mutations in EZH2 have been identified in follicular lymphoma (FL), and GCB diffuse large B cell lymphoma (DLBCL), leading to increased H3K27me3.[10−15] These findings suggest that dysregulation of H3K27me3, through EZH2 overexpression or point mutations, silences target genes important in tumor growth and survival. Together, these observations indicate that inhibition of EZH2 may be an attractive therapeutic approach for the treatment of cancer.

The inhibition of histone methyltransferase (HMT) catalytic activity by small molecules is an area of active research, and recent reports disclose small molecule inhibitors of several HMTs, including CARM1, DOT1L, EZH2, G9a/GLP, and Smyd2.[16−24] Herein we report the identification of highly potent, selective, small molecule inhibitors of EZH2, as exemplified by GSK926 (**3**) and GSK343 (**6**). Unlike deazaneplanocin (DZNep), which is thought to deplete PRC2 subunits in cancer cells by way of an indirect mechanism,[25] the compounds described here directly and selectively inhibit PRC2 enzymatic activity.

High-throughput screening of the GSK compound collection against EZH2 led to the identification of compound **1** (Table 1).[26] Biochemical mechanism of inhibition studies showed that **1** is competitive with the cofactor SAM and noncompetitive with peptide or nucleosome substrates. We selected **1** for optimization studies based on its activity and well-defined biochemical profile. Compounds **2**−**6** were synthesized as shown in Scheme 1.[27]

Preliminary structure−activity relationship (SAR) studies showed that the pyridone moiety, the 4,6-disubstitution pattern on the pyridone, the linking amide, and a branched alkyl group at the 1-position of the azaindazole core are all important features of the key pharmacophore (results from these studies will be published in a forthcoming manuscript). In contrast, the aza group at the 7-position of the azaindazole core could be removed without a loss of EZH2 inhibitory activity (**2**, $K_i^{app} = 74 \pm 11$ nM). The cyclopropyl group at the 6-position could be replaced by a

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Table 1. Data for Compounds 1−6

Compound	R_1	R_2	X	EZH2 K_i^{app} (nM)[a]	H3K27me3 IC_{50} (nM)[b]
1	CH_3	cyclopropyl	N	149 ± 28	10,632 ± 4905
2	CH_3	cyclopropyl	CH	74 ± 11	2,510 ± 960
3 (GSK926)	CH_3		CH	7.9 ± 3	324 ± 126
4	n-propyl		CH	0.60 ± 0.05	79 ± 7
5	CH_3		CH	14 ± 5	1,995 ± 1384
6 (GSK343)	n-propyl		CH	1.2 ± 0.2	174 ± 84

[a]IC_{50} values were measured at a high SAM concentration relative to K_m (7.5 μM). Apparent K_i values ± standard deviation were calculated using the Cheng−Prusoff relationship for a competitive inhibitor (n = 2). [b]H3K27me3 IC_{50} is the concentration of compound resulting in 50% inhibition of H3K27me3 in HCC1806 breast cancer cells normalized to total H3. Values are the mean of at least three independent replicates. See Supporting Information for assay conditions.

variety of substituents, and in particular, incorporation of an amine-containing group, attached to the 6-position of the core via an aryl linker, provided a significant boost in potency. For example, compounds **3** and **5**, which contain regioisomeric piperazinylpyridine groups at the 6-position, have EZH2 K_i^{app} = 7.9 ± 3 and 14 ± 5 nM, respectively (Table 1). Incorporation of an n-propyl group at the 4-position of the pyridone provided an additional boost in activity of at least 10-fold. For example, compounds **4** and **6**, which contain an n-propyl group at the 4-position of the pyridone, have EZH2 K_i^{app} = 0.60 ± 0.05 and 1.2 ± 0.2 nM, respectively.

To elucidate the mode of inhibition of these inhibitors, we determined IC_{50} values at eight SAM concentrations ranging from 3 μM to 15 μM, and then we replotted IC_{50} as a function of the $[SAM]/K_m$ ratio. The resulting pattern of increasing IC_{50} values with increasing $[SAM]/K_m$ is best explained by a model of inhibition where, for example, **6** is competitive with SAM (Figure 1). A SAM-competitive mechanism for **6** is consistent with the mechanism of inhibition data generated for the structurally similar molecule **1**.[26]

Since compound **6** (GSK343) is SAM-competitive, and many enzymes utilize SAM as a cofactor, we determined the selectivity of compounds **3**, **4**, and **6** against a panel of SAM-utilizing methyltransferases (Table 2). Overall, these compounds are highly selective for EZH2 over most other methyltransferases tested with selectivity greater than 1000-fold. The only exception is EZH1, where the selectivity of compounds **3**, **4**, and **6** is 125-, 25-, and 60-fold, respectively. EZH1 is highly homologous to EZH2 with 76% sequence identity overall and 96% sequence identity

Scheme 1. Synthesis of Indazole Derivatives 2−6[a]

7 → (a) → 9: R_1 = CH_3; 10: R_1 = nPr ← (b,c) ← 8

11 → (d) → 12 → (e) → 13 → (f) → 14 R_1 = CH_3; 15 R_1 = n-Pr → (g) →

2 R_1 = CH_3, R_2 = c-Pr
3 R_1 = CH_3, R_2 =
4 R_1 = n-Pr, R_2
5 R_1 = CH_3, R_2 =
6 R_1 = n-Pr, R_2 =

[a]Reagents and conditions: (a) (1) H_2 (100 psi), 10% Pd/C, Pt_2O, NaOAc, AcOH, 48 h; (2) HCl, EtOH, 2 h, 0 °C, (b) cyanoacetamide, KOtBu, DMSO, 30 min at RT then O_2, RT, 48 h; (c) H_2, (60 psi), Raney Ni, NH_4OH, RT, 48 h; (d) NaH (60% dispersion), 2-bromopropane, DMF, 0 °C to RT, 16 h; (e) 3 M NaOH, 4:1 MeOH/THF, RT, 3 h, then adjust to pH 4−5 with HCl; (f) **9** or **10**, EDC, HOAT, NMM, DMSO, RT, 12−48 h; (g) for **2**: cyclopropylboronic acid, $PdCl_2(dppf)\cdot CH_2Cl_2$, $NaHCO_3$, 3:1:1 dioxane/DMF/H_2O, 150 °C, 2 h; for **3**: arylboronate ester, $PdCl_2(dppf)\cdot CH_2Cl_2$, $NaHCO_3$, 3:1 DME/H_2O, microwave, 130 °C, 30 min; for **4**, **5**, and **6**: arylboronate ester, $PdCl_2(dppf)\cdot CH_2Cl_2$, $NaHCO_3$, 3:1 dioxane/H_2O, microwave, 110 °C, 20 min.

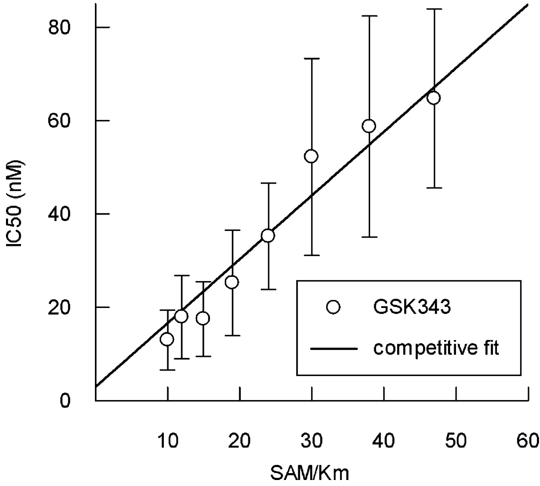

Figure 1. Mechanism of EZH2 inhibition for **6** (GSK343). The pattern of increasing IC_{50} with $[SAM]/K_m$ is best explained by a model of inhibition where **6** is competitive with SAM. The data were fit to models of competitive, uncompetitive, and noncompetitive inhibition and were best fit by a competitive model as determined by *F*-test analysis.

within the catalytic SET domain. The structural factors responsible for the selectivity over EZH1, and for the different levels of selectivity for EZH2 over EZH1, are unclear at this time. We also evaluated compounds **3**, **4**, and **6** in a broad screening panel of receptor and enzyme targets, and we found little to no cross-reactivity (Table 1 of the Supporting Information). Overall, these studies demonstrate that **3**, **4** and **6** are highly selective EZH2 inhibitors with good, albeit lower, selectivity over EZH1.

To evaluate cellular activity, we developed an immunofluorescent cell imaging assay to quantify nuclear H3K27me3 levels in the cancer cells. Incubation of HCC1806 breast cancer cells with the EZH2 inhibitors for 72 h led to dose-dependent reductions in nuclear H3K27me3 (Figure 2a,b). The cellular H3K27me3 IC_{50} values are shown in Table 1. At higher doses (>1000 nM), greater than 90% inhibition of global H3K27me3 was achieved with compounds **3** and **6**. These results show that

Table 2. Selectivity of 3, 4, and 6 vs Methyltransferases[a]

enzyme	3 IC_{50} (μM)	4 IC_{50} (μM)	6 IC_{50} (μM)
EZH2[b]	0.020	0.004	0.004
EZH1[b]	2.5	0.10	0.24
DNMT1	>100	121	>100
DNMT3a	>100	>100	>100
DNMT3b	>100	>100	>100
DOT1	>100	>100	>100
G9a	>100	>100	>100
MLL1			>100
MLL2			>100
MLL3			>100
MLL4			>100
PRMT1	>100	>100	>100
PRMT3	>100	54	74
PRMT4	>100	116	128
PRMT5	>100	>100	>100
SET7	214	49	63
SETMAR	>100	>100	>100
SUV39H1	>100	>100	>100
SUV39H2	>100	>100	123

[a] IC_{50} is the concentration of compound resulting in 50% inhibition of enzymatic activity. Values are the mean of at least two independent replicates. See Supporting Information for assay conditions. [b] Data generated using 5-component complex.

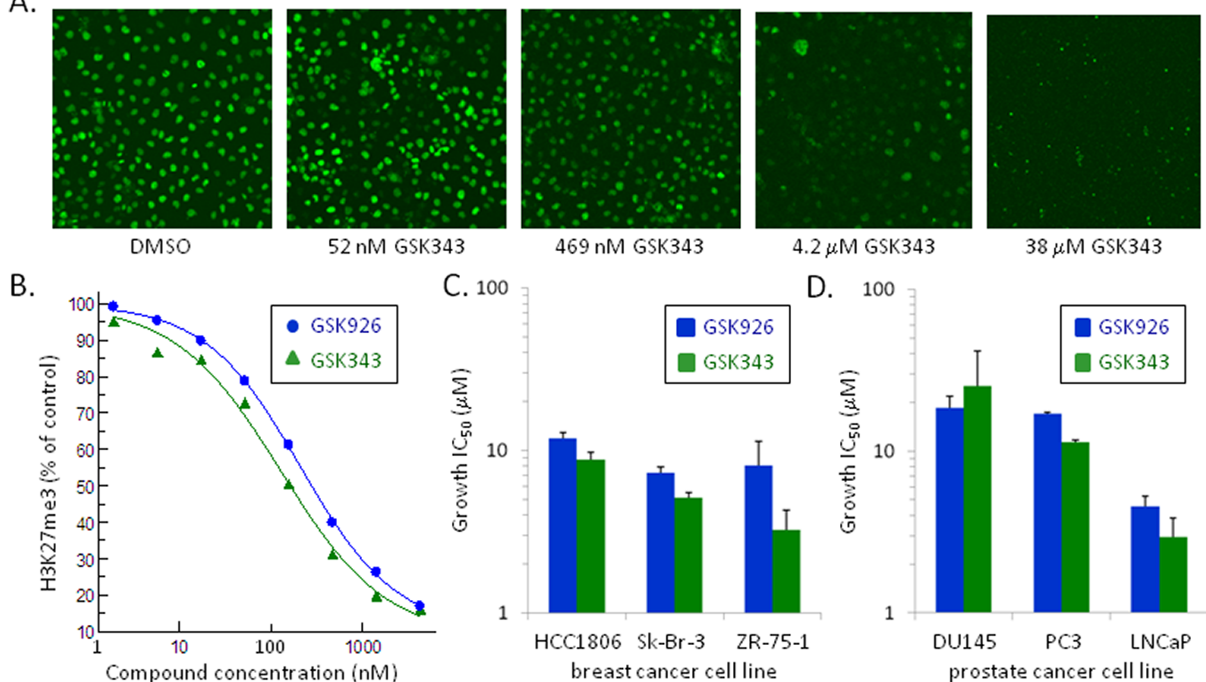

Figure 2. Biological characterization of **3** (GSK926) and **6** (GSK343). All data represent the average of at least two independent replicates, and error bars represent standard deviation. (a) H3K27me3 imaging in HCC1806 cells treated with DMSO or GSK343 for 72 h; (b) comparison of GSK926 and GSK343 in the HCC1806 imaging assay; (c) proliferation effects of GSK926 and GSK343 in breast cancer cells (6 days); (d) proliferation effects of GSK926 and GSK343 in prostate cancer cells (6 days).

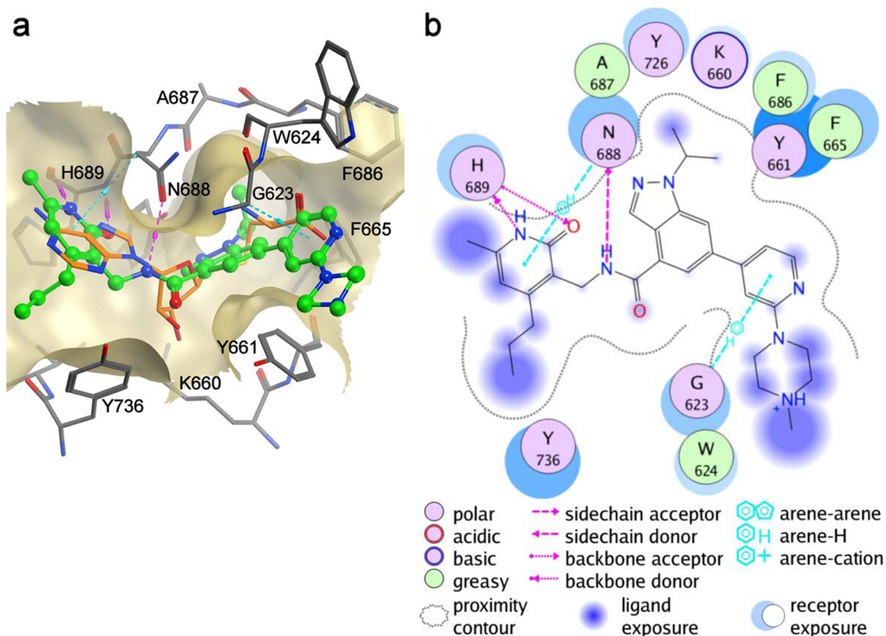

Figure 3. Proposed binding mode of **6** (GSK343). (a) EZH2 homology model, showing the proposed binding of **6** (green) relative to the binding of SAH (orange); (b) two-dimensional representation of the proposed binding mode of **6**.

compounds **3**−**6** are potent inhibitors of EZH2-mediated methylation in cells.

To evaluate the effect of EZH2 inhibition on cell proliferation, we chose several breast and prostate cancer cell lines, since studies have demonstrated that RNAi-mediated knockdown of EZH2 affects the growth of cancer cell lines derived from these tumor types[3,28,29] (Figure 2c,d). In this 6-day proliferation assay, **6** demonstrated slightly greater potency than **3** in all cell lines. This is consistent with the increased activity of **6** on EZH2 methyltransferase activity and suggests that the growth inhibition is related to EZH2 inhibition and not off-target effects. Among the cell lines evaluated in this study, the prostate cancer cell line LNCaP was the most sensitive to EZH2 inhibition, with growth IC_{50} values of 4.5 and 2.9 μM for **3** and **6**, respectively. These growth IC_{50} values are approximately 20-fold above the IC_{50} on H3K27me3, suggesting that nearly complete erasure of the H3K27me3 mark is required for growth inhibition. A roughly 10-fold range of growth inhibition potency was observed among the remaining cell lines, suggesting that there exists a spectrum of dependence on EZH2 activity. Additional studies will be required to evaluate whether prolonged exposure to **6** and **3** elicits more pronounced growth inhibition in these and other cell lines.

In rat PK studies, compounds **4** and **6** displayed high clearance. Consequently, although they are useful as *in vitro* tools to investigate EZH2 biology, they are not suitable for *in vivo* studies.

EZH2 is only active as part of the multicomponent PRC2 complex, and to date, no X-ray crystal structures of EZH2 or the PRC2 complex have been reported. Therefore, we developed an EZH2 homology model to determine a plausible binding mode for our inhibitors. To model the EZH2 SET domain (residues 600−726), we used the reported cocrystal structure of GLP (EHMT1) bound to an H3K9me2 peptide substrate (accession code 2RFI).[30] When compound **6** (GSK343) is docked into the SAM binding pocket (Figure 3), the pyridone N—H and C═O make hydrogen-bonding interactions with the backbone C═O and backbone N—H of His689, respectively. These interactions are the same as those made by the adenine moiety of SAM. The indazole ring makes hydrophobic contacts with Y661 and places the 1-position isopropyl group near the lysine tunnel adjacent to the SAM site. The piperazinylpyridine substituent projects toward solvent and makes hydrophobic contacts with Trp624, Phe686, and Phe685. The propyl at the 4-position of the pyridone also points toward solvent, but it appears to make hydrophobic contacts with Y736.

In summary, we have identified highly potent, selective, SAM-competitive, cell-active inhibitors of the histone methyltransferase EZH2. The present work adds to a growing body of evidence suggesting that histone methyltransferases are "druggable" targets. The compounds described herein, such as **3** (GSK926) and **6** (GSK343), are small molecule, *in vitro* chemical tools that should enable more detailed investigation into EZH2 biology, with the ultimate goal of identifying therapeutic agents for the treatment of human diseases.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details for the synthesis and characterization of compounds **1**−**6**, biochemical assays, the cell mechanistic assay, and the *in vitro* proliferation assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s): All authors are current or former employees of GlaxoSmithKline.

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■ REFERENCES

(1) Kouzarides, T. Chromatin Modifications and Their Function. *Cell* **2007**, *128*, 693−705.

(2) Jones, P. A.; Baylin, S. B. The Epigenomics of Cancer. *Cell* **2007**, *128*, 683−692.

(3) Wilson, C. B.; Rowell, E.; Sekimata, M. Epigenetic Control of T-Helper-Cell Differentiation. *Nat. Rev. Immunol.* **2009**, *9*, 91−105.

(4) Tsankova, N.; Renthal, W.; Kumar, A.; Nestler, E. J. Epigenetic Regulation in Psychiatric Disorders. *Nat. Rev. Neurosci.* **2007**, *8*, 355−367.

(5) Simon, J. A.; Lange, C. A. Roles of the EZH2 Histone Methyltransferase in Cancer Epigenetics. *Epigenetics Mutat. Res.* **2008**, *647*, 21−29.

(6) Varambally, S.; Dhanasekaran, S. M.; Zhou, M.; Barrette, T. R.; Kumar-Sinha, C.; Sanda, M. G.; Ghosh, D.; Pienta, K. J.; Sewalt, R. G. A. B.; 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.

(7) Kleer, C. G.; Cao, Q.; Varambally, S.; Shen, R.; Ota, I.; Tomlins, S. A.; Ghosh, D.; Sewalt, R. G. A. B.; 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.

(8) Wagener, N.; Macher-Goeppinger, S.; Pritsch, M.; Hüsing, J.; Hoppe-Seyler, K.; Schirmacher, P.; Pfitzenmaier, J.; Haferkamp, A.; Hoppe-Seyler, F.; Hohenfellner, M. Enhancer of Zeste Homolog 2 (EZH2) Expression is an Independent Prognostic Factor in Renal Cell Carcinoma. *BMC Cancer* **2010**, *10*, 524.

(9) Takawa, M.; Masuda, K.; Kunizaki, M.; Daigo, Y.; Takagi, K.; Iwai, Y.; Cho, H.-S.; Toyokawa, G.; Yamane, Y.; Maejima, K.; Field, H. I.; Kobayashi, T.; Akasu, T.; Sugiyama, M.; Tsuchiya, E.; Atomi, Y.; Ponder, B. A. J.; Nakamura, Y.; Hamamoto, R. Validation of the Histone Methyltransferase EZH2 as a Therapeutic Target for Various Types of Human Cancer and as a Prognostic Marker. *Cancer Sci.* **2011**, *102*, 1298−1305.

(10) Morin, R. D.; Johnson, N. A.; Severson, T. M.; Mungall, A. J.; An, J.; Goya, R.; Paul, J. E.; Boyle, M.; Woolcock, B. W.; Kuchenbauer, F.; Yap, D.; Humphries, R. K.; Griffith, O. L.; Shah, S.; Zhu, H.; Kimbara, M.; Shashkin, P.; Charlot, J. F.; Tcherpakov, M.; Corbett, R.; Tam, A.; Varhol, R.; Smailus, D.; Moksa, M.; Zhao, Y.; Delaney, A.; Qian, H.; Birol, I.; Schein, J.; Moore, R.; Holt, R.; Horsman, D. E.; Connors, J. M.; Jones, S.; Aparicio, S.; Hirst, M.; Gascoyne, R. D.; Marra, M. A. Somatic Mutations Altering EZH2 (Tyr641) in Follicular and Diffuse Large B-Cell Lymphomas of Germinal-Center Origin. *Nat. Genet.* **2010**, *42*, 181−185.

(11) Morin, R. D.; Mendez-Lago, M.; Mungall, A. J.; Goya, R.; Mungall, K. L.; Corbett, R. D.; Johnson, N. A.; Severson, T. M.; Chiu, R.; Field, M.; Jackman, S.; Krzywinski, M.; Scott, D. W.; Trinh, D. L.; Tamura-Wells, J.; Li, S.; Firme, M. R.; Rogic, S.; Griffith, M.; Chan, S.; Yakovenko, O.; Meyer, I. M.; Zhao, E. Y.; Smailus, D.; Moksa, M.; Chittaranjan, S.; Rimsza, L.; Brooks-Wilson, A.; Spinelli, J. J.; Ben-Neriah, S.; Meissner, B.; Woolcock, B.; Boyle, M.; McDonald, H.; Tam, A.; Zhao, Y.; Delaney, A.; Zeng, T.; Tse, K.; Butterfield, Y.; Birol, I.; Holt, R.; Schein, J.; Horsman, D. E.; Moore, R.; Jones, S. J. M.; Connors, J. M.; Hirst, M.; Gascoyne, R. D.; Marra, M. A. Frequent Mutation of Histone-Modifying Genes in Non-Hodgkin Lymphoma. *Nature* **2011**, *476*, 298−303.

(12) Pasqualucci, L.; Trifonov, V.; Fabbri, G.; Ma, J.; Rossi, D.; Chiarenza, A.; Wells, V. A.; Grunn, A.; Messina, M.; Elliot, O.; Chan, J.; Bhagat, G.; Chadburn, A.; Gaidano, G.; Mullighan, C. G.; Raul Rabadan, R.; Riccardo Dalla-Favera, R. Analysis of the Coding Genome of Diffuse Large B-Cell Lymphoma. *Nat. Genet.* **2011**, *43*, 830−837.

(13) Ernst, T.; Chase, A. J.; Score, J.; Hidalgo-Curtis, C. E.; Bryant, C.; Jones, A. V.; Waghorn, K.; Zoi, K.; Ross, F. M.; Reiter, A.; Hochhaus, A.; Drexler, H. G.; Duncombe, A.; Cervantes, F.; Oscier, D.; Boultwood, J.; Grand, F. H.; Cross, N. C. P. Inactivating Mutations of the Histone Methyltransferase Gene EZH2 in Myeloid Disorders. *Nat. Genet.* **2010**, *42*, 722−726.

(14) Chang, C. J.; Hung, M.-C. The Role of EZH2 in Tumour Progression. *Br. J. Cancer* **2012**, *106*, 243−247.

(15) 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.; Chen, S. B.; Della Pietra, A., III; Dul, E.; Hughes, A. M.; Gilbert, S. A.; Thrall, S. H.; Tummino, P. J.; Kruger, R. G.; Brandt, M.; Schwartz, B.; Creasy, C. L. 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.

(16) Allan, M.; Manku, S.; Therrien, E.; Nguyen, N.; Styhler, S.; Robert, M.-F.; Goulet, A.-C.; Petschner, A. J.; Rahil, G.; MacLeod, A. R.; Déziel, R.; Besterman, J. M.; Nguyen, H.; Wahhab, A. N-Benzyl-1-Heteroaryl-3-(trifluoromethyl)-1H-Pyrazole-5-Carboxamides as Inhibitors of Co-activator Associated Arginine Methyltransferase 1 (CARM1). *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1218−1223.

(17) Liu, F.; Chen, X.; Allali-Hassani, A.; Quinn, A. M.; Wasney, G. A.; Dong, A.; Barsyte, D.; Kozieradzki, I.; Senisterra, G.; Chau, I.; Siarheyeva, A.; Kireev, D. B.; Jadhav, A.; Herold, J. M.; Frye, S. V.; Arrowsmith, C. H.; Brown, P. J.; Simeonov, A.; Vedadi, M.; Jin, J. Discovery of a 2,4-Diamino-7-Aminoalkoxyquinazoline as a Potent and Selective Inhibitor of Histone Lysine Methyltransferase G9a. *J. Med. Chem.* **2009**, *52*, 7950−7953.

(18) Liu, F.; Chen, X.; Allali-Hassani, A.; Quinn, A. M.; Wigle, T. J.; Wasney, G. A.; Dong, A.; Senisterra, G.; Chau, I.; Siarheyeva, A.; Norris, J. L.; Kireev, D. B.; Jadhav, A.; Herold, J. M.; Janzen, W. P.; Arrowsmith, C. H.; Frye, S. V.; Brown, P. J.; Simeonov, A.; Vedadi, M.; Jin, J. Protein Lysine Methyltransferase G9a Inhibitors: Design, Synthesis, and Structure Activity Relationships of 2,4-Diamino-7-Aminoalkoxy-Quinazolines. *J. Med. Chem.* **2010**, *53*, 5844−5857.

(19) Daigle, S. R.; Olhava, E. J.; Therkelsen, C. A.; Majer, C. R.; Sneeringer, C. J.; Song, J.; Johnston, L. D.; Porter Scott, M.; Smith, J. J.; Xiao, Y.; Jin, L.; Kuntz, K. W.; Chesworth, R.; Moyer, M. P.; Bernt, K. M.; Tseng, J.-C.; Kung, A. L.; Armstrong, S. A.; Copeland, R. A.; Richon, V. M.; Pollock, R. M. Selective Killing of Mixed Lineage Leukemia Cells by a Potent Small-Molecule DOT1L Inhibitor. *Cancer Cell* **2011**, *20*, 53−65.

(20) Yao, Y.; Chen, P.; Diao, J.; Cheng, G.; Deng, L.; Anglin, J. L.; Prasad, B. V. V; Song, Y. Selective Inhibitors of Histone Methyltransferase DOT1L: Design, Synthesis, and Crystallographic Studies. *J. Am. Chem. Soc.* **2011**, *133*, 16746−16749.

(21) Ferguson, A. D.; Larsen, N. A.; Howard, T.; Pollard, H.; Green, I.; Grande, C.; Cheung, T.; Garcia-Arenas, R.; Cowen, S.; Wu, J.; Godin, R.; Chen, H.; Keen, N. Structural Basis of Substrate Methylation and Inhibition of SMYD2. *Structure* **2011**, *19*, 1262−1273.

(22) Vedadi, M.; Barsyte-Lovejoy, D.; Liu, F.; Rival-Gervier, S.; Allali-Hassani, A.; Labrie, V.; Wigle, T. J.; DiMaggio, P. A.; Wasney, G. A.; Siarheyeva, A.; Dong, A.; Tempel, W.; Wang, S-C.; Chen, X.; Chau, I.; Mangano, T. J.; Huang, X-P.; Simpson, C. D.; Pattenden, S. G.; Norris, J. G.; Kireev, D. B.; Tripathy, A.; Edwards, A.; Roth, B. L.; Janzen, W. P.; Garcia, B. A.; Petronis, A.; Ellis, J.; Brown, P. J.; Frye, S. V.; Arrowsmith, C. H.; Jin, J. A Chemical Probe Selectively Inhibits G9a and GLP Methyltransferase Activity in Cells. *Nat. Chem. Biol.* **2011**, *7*, 566−574.

(23) McCabe, M. T.; Ott, H. M.; Ganji, G.; Korenchuk S.; Thompson, C. S.; Van Aller, G. S.; Liu, Y.; Graves A. P.; Della Pietra III, A.; 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. T.; Creasy, C. L. Inhibition of EZH2Methyltransferase Activity as a Therapeutic Strategy for Diffuse Large B-Cell Lymphoma with EZH2 Activating Mutations. *Nature*, published online October 10, **2012**; DOI: 10.1038/nature11606.

(24) 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.*, published online September 30, **2012**; DOI: 10.1038/NCHEMBIO.1084.

(25) Miranda, T. B.; Cortez, C. C.; Yoo, C. B.; Liang, G.; Abe, M.; Kelly, T. K.; Marquez, V. E.; Jones, P. A. DZNep is a Global Histone

Methylation Inhibitor that Reactivates Developmental Genes not Silenced by DNA Methylation. *Mol. Cancer Ther.* **2009**, *8*, 1579−1588.

(26) 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.; 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*, published online August 17, **2012**. DOI: 10.1177/1087057112453765.

(27) Duquenne, C.; Johnson, N.; Knight, S. D.; LaFrance, L.; Miller, W. H; Newlander, K.; Romeril, S.; Rouse, M. B.; Tian, X. R.; Verma, S. K. Indazoles. PCT Int. Appl. (2011), WO 2011140325, A1 20111110.

(28) Bryant, R. J.; Cross, N. A.; Eaton, C. L.; Hamdy, F. C.; Cunliffe, V. T. EZH2 promotes proliferation and invasiveness of prostate cancer cells. *Prostate* **2007**, *67*, 547−556.

(29) Gonzalez, M. E.; Li, X.; Toy, K.; DuPrie, M.; Ventura, A. C.; Banerjee, M.; Ljungman, M.; Merajver, S. D.; Kleer, C. G. Down-regulation of EZH2 decreases growth of estrogen receptor negative invasive breast carcinoma and requires BRCA1. *Oncogene* **2009**, *28*, 843−853.

(30) Wu, H.; Min, J.; Lunin, V. V.; Antoshenko, T.; Dombrovski, L.; Zeng, H.; Allali-Hassani, A.; Campagna-Slater, V.; Vedadi, M.; Arrowsmith, C. H.; Plotnikov, A. N.; Schapira, M. Structural Biology of Human H3K9Methyltransferases. *PLoS One* **2010**, *5*, e8570.