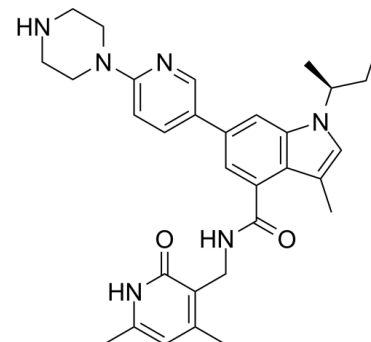


## Data Sheet

<b>Product Name:</b>	GSK126
<b>Cat. No.:</b>	CS-1401
<b>CAS No.:</b>	1346574-57-9
<b>Molecular Formula:</b>	C <sub>31</sub> H <sub>38</sub> N <sub>6</sub> O <sub>2</sub>
<b>Molecular Weight:</b>	526.67
<b>Target:</b>	Histone Methyltransferase
<b>Pathway:</b>	Epigenetics
<b>Solubility:</b>	DMSO : 12.5 mg/mL (23.73 mM; Need ultrasonic); H <sub>2</sub> O : < 0.1 mg/mL (insoluble)



### BIOLOGICAL ACTIVITY:

GSK126 (GSK2816126A) is a potent, highly selective inhibitor of **EZH2 methyltransferase** with an **IC<sub>50</sub>** of 9.9 nM. **IC<sub>50</sub> & Target:** IC<sub>50</sub>: 9.9 nM (EZH2)<sup>[1]</sup> **In Vitro:** GSK126 potently inhibits both wild-type and mutant EZH2 methyltransferase activity with similar potencies ( $K_i$ =0.5-3 nM) independent of substrate used, and is competitive with S-adenosyl-methionine (SAM) and non-competitive with peptide substrates. GSK126 is highly selective against other methyltransferases and multiple other protein classes (EZH1, IC<sub>50</sub>=680 nM)<sup>[1]</sup>. Treatment of three SCLC cell lines with GSK126, induces growth inhibition. SCLC cell lines (Lu130, H209, and DMS53) are treated with 0.5, 2, and 8  $\mu$ M GSK126, and growth curve is analyzed by WST-8 assay. Inhibition of cellular growth by GSK126 treatment is observed at 8  $\mu$ M in all the three cell lines, while Lu130 and H209 are more sensitive to GSK126, even at lower doses<sup>[2]</sup>. **In Vivo:** GSK126 is administered intraperitoneally at a dose volume of 0.2 mL per 20 g body weight in female beige SCID mice. GSK126 effectively inhibits the proliferation of EZH2 mutant DLBCL cell lines and markedly inhibits the growth of EZH2 mutant DLBCL xenografts in mice<sup>[1]</sup>.

### PROTOCOL (Extracted from published papers and Only for reference)

**Kinase Assay:** <sup>[1]</sup>The five-member PRC2 complex (Flag-EZH2, EED, SUZ12, AEBP2, RbAp48) containing either wild-type or mutant (A677G, Y641N, Y641C, Y641H, Y641S or Y641F) EZH2 is prepared. GSK126 is dissolved in DMSO and tested at concentrations of 0.6 nM to 300 nM with a final DMSO concentration of 2.5%. In contrast to wild-type EZH2 which prefers H3K27me0 as a substrate in vitro, EZH2 Y641 mutants prefer H3K27me2 and have little activity with H3K27me0 or H3K27me1. The A677G mutant is distinct from both the wild-type and Y641 mutant forms of EZH2 in that it efficiently methylates H3K27me0, H3K27me1, and H3K27me2; therefore, histone H3 peptides (residues 21-44; 10  $\mu$ M final) with either K27me0 (wild type, A677G EZH2), K27me1 (A677G EZH2), or K27me2 (A677G, Y641N, Y641C, Y641H, Y641S and Y641F EZH2) are used as methyltransferase substrates. GSK126 is added to plates followed by addition of 6 nM EZH2 complex and peptide. As the potency of GSK126 is at or near the tight binding limit of an assay run at  $[SAM]=K_m$ , IC<sub>50</sub> values are measured at a high concentration of the competitive substrate SAM relative to its  $K_m$  (7.5  $\mu$ M SAM where the SAM  $K_m$  is 0.3  $\mu$ M)<sup>[1]</sup>. **Cell Assay:** GSK126 is dissolved in DMSO (10 mM) and stored, and then diluted with appropriate media before use<sup>[2]</sup>. <sup>[2]</sup>JUB- and PTRF-introduced DMS53 cells are seeded at density of  $1 \times 10^3$  cells/well in 96-well plate, and cellular growth is analyzed using WST-8 kit at 12, 36, 60, and 84 h. Cellular growth of Lu130, H209, and DMS53 with treatment by DZNep or GSK126 is also analyzed using WST-8 kit. DZNep is dissolved in PBS at 5 mM, and cells are cultured at the final concentration of 5  $\mu$ M. GSK126 is dissolved in DMSO at 10 mM, and cells are cultured at 0.5, 2, and 8  $\mu$ M<sup>[2]</sup>. **Animal Administration:** GSK126 is dissolved in 20% captisol adjusted to pH 4-4.5 with 1 N acetic acid<sup>[1]</sup>. <sup>[1]</sup>Mice<sup>[1]</sup>

GSK126 or vehicle is administered intraperitoneally at a dose volume of 0.2 mL per 20 g body weight. Pfeiffer or KARPAS-422 cells ( $1 \times 10^7$ ) in 100% Matrigel are implanted subcutaneously in female beige SCID mice. Tumors are measured with calipers, and block randomized according to tumour size into treatment groups. For efficacy studies, 10 mice are randomized in each treatment group before the initiation of dosing and GSK126 treatment is initiated once the tumour volumes are approximately 200 mm<sup>3</sup> in the Pfeiffer

and KARPAS-422 studies and 500 mm<sup>3</sup> in the KARPAS-422 intermittent dosing study. Mice are weighed and tumors measured with calipers twice weekly. Two-tailed t-tests are conducted assuming two samples of equal variance.

#### References:

- [1]. McCabe MT, et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. Nature. 2012 Dec 6;492(7427):108-12.
- [2]. Sato T, et al. PRC2 overexpression and PRC2-target gene repression relating to poorer prognosis in small cell lung cancer. Sci Rep. 2013 May 29;3:1911.

#### CAIndexNames:

1H-Indole-4-carboxamide, N-[(1,2-dihydro-4,6-dimethyl-2-oxo-3-pyridinyl)methyl]-3-methyl-1-[(1S)-1-methylpropyl]-6-[6-(1-piperazinyl)-3-pyridinyl]-

#### SMILES:

O=C(C1=CC(C2=CC=C(N3CCNCC3)N=C2)=CC4=C1C(C)=CN4[C@@H](C)CC)NCC5=C(C)C=C(C)NC5=O

**Caution: Product has not been fully validated for medical applications. For research use only.**

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