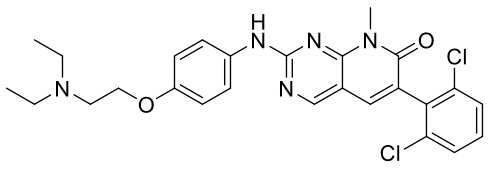
Product data sheet

MedKoo Cat#: 406345 Name: PD0166285 CAS#: 185039-89-8 Chemical Formula: $C_{26}H_{27}Cl_2N_5O_2$ Exact Mass: 511.15418 Molecular Weight: 512.43	
Product supplied as:	Powder
Purity (by HPLC):	≥ 98%
Shipping conditions	Ambient temperature
Storage conditions:	Powder: -20°C 3 years; 4°C 2 years.
	In solvent: -80°C 3 months; -20°C 2 weeks.

1. Product description:
PD0166285 is a potent Wee1 inhibitor and Chk1 inhibitor with activity at nanomolar concentrations. This G2 checkpoint abrogation by PD0166285 was demonstrated to kill cancer cells, there at a toxic highest dose of 0.5 muM in some cell lines for exposure periods of no longer than 6 hours. The deregulated cell cycle progression may have ultimately damaged the cancer cells. We herein report one of the mechanism by which PD0166285 leads to cell death in the B16 mouse melanoma cell line.

2. CoA, QC data, SDS, and handling instruction
SDS and handling instruction, CoA with copies of QC data (NMR, HPLC and MS analytical spectra) can be downloaded from the product web page under "QC And Documents" section. Note: copies of analytical spectra may not be available if the product is being supplied by MedKoo partners. Whether the product was made by MedKoo or provided by its partners, the quality is 100% guaranteed.

3. Solubility data

Solvent	Max Conc. mg/mL	Max Conc. mM
DMSO	60.0	117.1

4. Stock solution preparation table:

Concentration / Solvent Volume / Mass	1 mg	5 mg	10 mg
1 mM	1.95 mL	9.76 mL	19.51 mL
5 mM	0.39 mL	1.95 mL	3.90 mL
10 mM	0.20 mL	0.98 mL	1.95 mL
50 mM	0.04 mL	0.20 mL	0.39 mL

5. Molarity Calculator, Reconstitution Calculator, Dilution Calculator
Please refer the product web page under section of "Calculator"

6. Recommended literature which reported protocols for in vitro and in vivo study
In vitro study

1. Hashimoto O, Shinkawa M, Torimura T, Nakamura T, Selvendiran K, Sakamoto M, Koga H, Ueno T, Sata M. Cell cycle regulation by the Wee1 inhibitor PD0166285, pyrido [2,3-d] pyimidine, in the B16 mouse melanoma cell line. BMC Cancer. 2006 Dec 19;6:292. doi: 10.1186/1471-2407-6-292. PMID: 17177986; PMCID: PMC1770931.

2. Wang Y, Li J, Booher RN, Kraker A, Lawrence T, Leopold WR, Sun Y. Radiosensitization of p53 mutant cells by PD0166285, a novel G(2) checkpoint abrogator. Cancer Res. 2001 Nov 15;61(22):8211-7. PMID: 11719452.

In vivo study

1. Mir SE, De Witt Hamer PC, Krawczyk PM, Balaj L, Claes A, Niers JM, Van Tilborg AA, Zwinderman AH, Geerts D, Kaspers GJ, Peter Vandertop W, Cloos J, Tannous BA, Wesseling P, Aten JA, Noske DP, Van Noorden CJ, Würdinger T. In silico analysis of kinase expression identifies WEE1 as a gatekeeper against mitotic catastrophe in glioblastoma. Cancer Cell. 2010 Sep 14;18(3):244-57. doi: 10.1016/j.ccr.2010.08.011. PMID: 20832752; PMCID: PMC3115571.

7. Bioactivity
Biological target:

Product data sheet

PD0166285 is a potent Wee1 and Chk1 inhibitor with activity at nanomolar concentrations (IC50=24 nM for Wee1 and 72 nM for Myt1).

In vitro activity

B16 cells dramatically abrogated the G2 checkpoint and were found to arrest in the early G1 phase by treatment with 0.5 muM for 4 hours observed by flow cytometry. Cyclin D mRNA decreased within 4 hours observed by Real-time PCR. Rb was dephosphrylated for 24 hours. However, B16 cells did not undergo cell death after 0.5 muM treatment for 24 hours. Immnofluoscence microscopy showed that the cells become round and small in the morphogenesis. More interesting phenomena were that microtubule stabilization was blocked, and Wee1 distribution was restricted after treatment for 4 hours.

Reference: BMC Cancer. 2006 Dec 19;6:292. https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/17177986/

In vivo activity

To determine the pharmacological characteristics and anti-tumor efficacy of PD0166285 on GBM outcome in vivo, GBM cells were first implanted subcutaneously (SC) into nude mice and determine the PD0166285 dosage sufficient to inhibit WEE1 activity in vivo. Mice were injected with various doses of PD0166285 (0, 20, 100, 200, or 400 µM in 100 µl) 20 days after tumor cells implantation. No adverse side effects were observed at the concentrations used. Tumors were removed 24 hr later and analyzed for inhibition of WEE1-mediated CDC2 phosphorylation by western blotting (Figure S4B). A single injection of 20 µM of the WEE1 inhibitor considerably reduced the CDC2 phosphorylation and was used in further experiments. Next, U251-FM human GBM cells were injected intracranially into nude mice. Mice with established GBMs were treated daily with PD0166285 or phosphate buffer solution (PBS) control via an intraperitoneal (IP) injection starting at day 14 after injection of the GBM cells. At day 15, the mice were sham irradiated or exposed to a single dose of 6 Gy. The results showed strong tumor progression in both irradiated and non-irradiated mock treated mice at 6 weeks after injection of the cells (Figures 6E and 6F). Similarly, the non-irradiated PD0166285 treated mice showed strong increase in tumor signal after 6 weeks. In contrary, irradiated mice treated with PD0166285 showed significant tumor regression 6 weeks after tumor injection (Figures 6E and 6F). Additionally, tumor burden was markedly reduced in this animal group (Figure 6G). Survival analysis showed a significant ($p = 0.001$) advantage for combining irradiation with PD0166285 (Figure 6H). These results indicate that pharmacological targeting of WEE1 sensitizes U251-FM GBM tumors to IR in vivo.

Reference: Cancer Cell. 2010 Sep 14;18(3):244-57. https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/20832752/