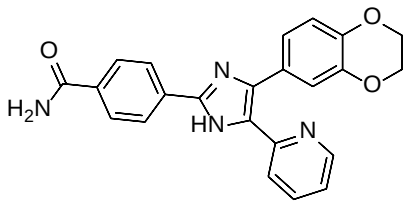
Product data sheet

MedKoo Cat#: 526754 Name: D-4476 CAS#: 301836-43-1 Chemical Formula: $C_{23}H_{18}N_4O_3$ Exact Mass: 398.1379 Molecular Weight: 398.42	
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:
D-4476, also known as Casein Kinase I Inhibitor, is a potent, selective and cell-permeant inhibitor of casein kinase 1 (CK1; IC50 = 200 nM from S. pombe,1 300 nM for CK1δ2). D4476 suppresses the site-specific phosphorylation and nuclear exclusion of FOXO1a. D4476 specifically inhibits the phosphorylation of endogenous forkhead box transcription factor O1a (FOXO1a) on Ser322 and Ser325 within its MPD, without affecting the phosphorylation of other sites.

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	49.71	124.77
DMSO:PBS (pH 7.2) (1:10)	0.10	0.25
DMF	30.0	75.30
Ethanol	14.96	37.55

4. Stock solution preparation table:

Concentration / Solvent Volume / Mass	**1 mg**	**5 mg**	**10 mg**
1 mM	2.51 mL	12.55 mL	25.10 mL
5 mM	0.50 mL	2.51 mL	5.02 mL
10 mM	0.25 mL	1.25 mL	2.51 mL
50 mM	0.05 mL	0.25 mL	0.50 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. Rena G, Bain J, Elliott M, Cohen P. D4476, a cell-permeant inhibitor of CK1, suppresses the site-specific phosphorylation and nuclear exclusion of FOXO1a. EMBO Rep. 2004 Jan;5(1):60-5. doi: 10.1038/sj.embor.7400048. PMID: 14710188; PMCID: PMC1298959.
2. Xu W, Huang Z, Gan Y, Chen R, Huang Y, Xue B, Jiang S, Yu Z, Yu K, Zhang S. Casein kinase 1α inhibits p53 downstream of MDM2-mediated autophagy and apoptosis in acute myeloid leukemia. Oncol Rep. 2020 Nov;44(5):1895-1904. doi: 10.3892/or.2020.7760. Epub 2020 Sep 9. PMID: 32901886; PMCID: PMC7550986.

In vivo study

1. Liu G, Li H, Zhang W, Yu J, Zhang X, Wu R, Niu M, Liu X, Yu R. Csnk1a1 inhibition modulates the inflammatory secretome and enhances response to radiotherapy in glioma. J Cell Mol Med. 2021 Jul 3. doi: 10.1111/jcmm.16767. Epub ahead of print. PMID: 34216174.

Product data sheet

7. Bioactivity

Biological target: D4476 is an inhibitor of casein kinase 1(CK1) with an IC50 value of 0.3 μM.

In vitro activity

To investigate the role of CK1α in AML (acute myeloid leukemia) cells, CK1α was inhibited using D4476. D4476 significantly reduced the cell viability of three AML cell lines HL-60, HEL and THP-1 in a time- and dose-dependent manner (Fig. 2A), while only a dose-dependent manner was shown after treatment with D4476 lower than 10 μM in patient blast cells. Whether D4476 affects the colony formation ability of AML cells was further investigated, and it was found that D4476 at 40 μM dramatically impaired the colony formation, as indicated with colony number and total cell number, in both HL-60 and HEL cells (Fig. 2B). Notably, there were many small cell clusters, but did not reach the standard of a colony in the D4476 treatment group in these two cell lines (Fig. 2B). Furthermore, D4476 dramatically induced apoptosis in a dose-dependent manner in three AML cell lines tested as well as patient blast cells (Fig. 3A). D4476 treatment promoted the cleavage of PARP, a classical marker of apoptosis, and decreased the expression of survivin, a negative marker protein of apoptosis, in HL-60, THP-1 and HEL cells as well as in patient blast cells (Fig. 3A).

Reference: Oncol Rep. 2020 Nov;44(5):1895-1904. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7550986/

In vivo activity

In order determine the therapeutic effect of D4476 in vivo in a clinically relevant model of GBM (glioblastoma multiforme), a glioma stem cell (GSC)-driven xenograft tumour mouse model was used. The tumour size in treatment with D4476 was smaller than those in the control group. Therefore, D4476-treated mice showed increased survival rate. Significantly, D4476 treatment combined with radiotherapy obviously inhibited the growth of tumour (Figure 6C). The survival time of mice bearing GBM was significantly increased in the combination group (Figure 6D). Compared with the control group, the median survival time of mice in the D4476 treatment group was extended by 8 days; radiotherapy treatment extended survival by 6 days, and D4476 combined with radiotherapy resulted in a 22-day extension (Table S3).

Reference: J Cell Mol Med. 2021 Jul 3. https://onlinelibrary.wiley.com/doi/10.1111/jcmm.16767