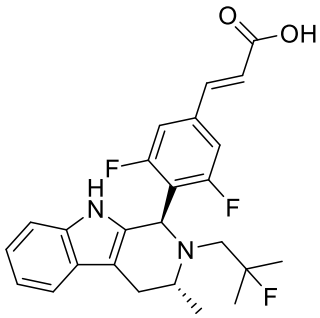
Product data sheet

MedKoo Cat#: 206208 Name: AZD9496 CAS#: 1639042-08-2 (free base) Chemical Formula: $C_{25}H_{25}F_3N_2O_2$ Exact Mass: 442.18681 Molecular Weight: 442.48	
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:
AZD9496 is a potent and orally bioavailable selective estrogen receptor downregulator and antagonist. AZD9496 can induce ERα degradation in breast cancer cell lines at picomolar concentrations. AZD9496 is curently under clinical trials. AZD9496 may be useful for the treatment of ER+ breast cancer.

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	88	198.88
Ethanol	88	198.88

4. Stock solution preparation table:

Concentration / Solvent Volume / Mass	1 mg	5 mg	10 mg
1 mM	2.26 mL	11.30 mL	22.60 mL
5 mM	0.45 mL	2.26 mL	4.52 mL
10 mM	0.23 mL	1.13 mL	2.26 mL
50 mM	0.05 mL	0.23 mL	0.45 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. De Savi C, Bradbury RH, Rabow AA, Norman RA, de Almeida C, Andrews DM, Ballard P, Buttar D, Callis RJ, Currie GS, Curwen JO, Davies CD, Donald CS, Feron LJ, Gingell H, Glossop SC, Hayter BR, Hussain S, Karoutchi G, Lamont SG, MacFaul P, Moss TA, Pearson SE, Tonge M, Walker GE, Weir HM, Wilson Z. Optimization of a Novel Binding Motif to (E)-3-(3,5-Difluoro-4-((1R,3R)-2-(2-fluoro-2-methylpropyl)-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylic Acid (AZD9496), a Potent and Orally Bioavailable Selective Estrogen Receptor Downregulator and Antagonist. J Med Chem. 2015 Oct 22;58(20):8128-40. doi: 10.1021/acs.jmedchem.5b00984. Epub 2015 Oct 7. PMID: 26407012.

2. Weir HM, Bradbury RH, Lawson M, Rabow AA, Buttar D, Callis RJ, Curwen JO, de Almeida C, Ballard P, Hulse M, Donald CS, Feron LJ, Karoutchi G, MacFaul P, Moss T, Norman RA, Pearson SE, Tonge M, Davies G, Walker GE, Wilson Z, Rowlinson R, Powell S, Sadler C, Richmond G, Ladd B, Pazolli E, Mazzola AM, D'Cruz C, De Savi C. AZD9496: An Oral Estrogen Receptor Inhibitor That Blocks the Growth of ER-Positive and ESR1-Mutant Breast Tumors in Preclinical Models. Cancer Res. 2016 Jun 1;76(11):3307-18. doi: 10.1158/0008-5472.CAN-15-2357. Epub 2016 Mar 28. PMID: 27020862.

In vivo study

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1. De Savi C, Bradbury RH, Rabow AA, Norman RA, de Almeida C, Andrews DM, Ballard P, Buttar D, Callis RJ, Currie GS, Curwen JO, Davies CD, Donald CS, Feron LJ, Gingell H, Glossop SC, Hayter BR, Hussain S, Karoutchi G, Lamont SG, MacFaul P, Moss TA, Pearson SE, Tonge M, Walker GE, Weir HM, Wilson Z. Optimization of a Novel Binding Motif to (E)-3-(3,5-Difluoro-4-((1R,3R)-2-(2-fluoro-2-methylpropyl)-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylic Acid (AZD9496), a Potent and Orally Bioavailable Selective Estrogen Receptor Downregulator and Antagonist. J Med Chem. 2015 Oct 22;58(20):8128-40. doi: 10.1021/acs.jmedchem.5b00984. Epub 2015 Oct 7. PMID: 26407012.

2. Weir HM, Bradbury RH, Lawson M, Rabow AA, Buttar D, Callis RJ, Curwen JO, de Almeida C, Ballard P, Hulse M, Donald CS, Feron LJ, Karoutchi G, MacFaul P, Moss T, Norman RA, Pearson SE, Tonge M, Davies G, Walker GE, Wilson Z, Rowlinson R, Powell S, Sadler C, Richmond G, Ladd B, Pazolli E, Mazzola AM, D'Cruz C, De Savi C. AZD9496: An Oral Estrogen Receptor Inhibitor That Blocks the Growth of ER-Positive and ESR1-Mutant Breast Tumors in Preclinical Models. Cancer Res. 2016 Jun 1;76(11):3307-18. doi: 10.1158/0008-5472.CAN-15-2357. Epub 2016 Mar 28. PMID: 27020862.

7. Bioactivity

Biological target:

AZD9496 is a potent and selective estrogen receptor (ERα) antagonist with an IC50 of 0.28 nM.

In vitro activity

To measure the rate at which AZD9496 downregulated ERα, SILAC experiments were performed in MCF-7 cells using mass spectroscopy analysis to detect ERα protein levels after treatment with compounds. Addition of AZD9496 increased the rate of degradation of the isotope-labeled ERα compared with DMSO control. Levels of newly synthesized ERα peptide were also reduced following AZD9496 and fulvestrant treatment, presumably due to ongoing degradation of newly synthesized ERα protein by compound present, whereas ERα protein levels continued to increase over time in the presence of tamoxifen or DMSO (Fig. 2). The t1/2 of ERα was decreased from 3 hours, in the presence of DMSO, to 0.75 hours with 100 nmol/L AZD9496 and 0.6 hours with 100 nmol/L fulvestrant (Supplementary Fig. S2). Downregulation of ER occurs through the 26S proteosomal pathway as no decrease in ERα protein level in MCF-7 cells was seen in the presence of the proteosome inhibitor MG132 (Supplementary Fig. S3). In addition, ERα downregulation was shown to be reversible in compound wash-out experiments, where ERα levels increased in a time-dependent manner back to basal levels over a 48-hour period following compound removal (Supplementary Fig. S4).

Reference: Cancer Res. 2016 Jun 1;76(11):3307-18. http://cancerres.aacrjournals.org/cgi/pmidlookup?view=long&pmid=27020862

In vivo activity

To test whether AZD9496 could act as a partial agonist in other tissues, agonist effects were measured in vivo in a previously validated immature female rat model designed to detect agonistic properties of test compounds by measuring increases in uterine weight. AZD9496, given once daily orally at 5 and 25 mg/kg produced statistically significant increases in uterine weight compared with the fulvestrant control ($P < 0.001$) but significantly lower than tamoxifen ($P = 0.001$; Fig. 3B). Histologic staining of uterine tissue samples also showed that the lengthening of the endometrial cells in the rat uteri appeared to have decreased compared with tamoxifen (Fig. 3C). In further studies, ERα protein levels were reduced (Fig. 3D).

Reference: Cancer Res. 2016 Jun 1;76(11):3307-18. http://cancerres.aacrjournals.org/cgi/pmidlookup?view=long&pmid=27020862