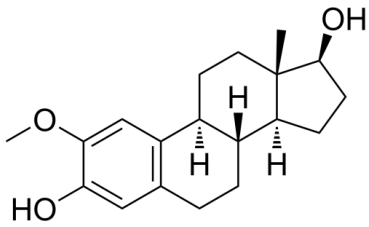
Data Sheet

Product Name:	2-Methoxyestradiol
Cat. No.:	CS-0176
CAS No.:	362-07-2
Molecular Formula:	$C_{19}H_{26}O_3$
Molecular Weight:	302.41
Target:	Apoptosis; Autophagy; Endogenous Metabolite; Microtubule/Tubulin; Reactive Oxygen Species
Pathway:	Apoptosis; Autophagy; Cell Cycle/DNA Damage; Cytoskeleton; Immunology/Inflammation; Metabolic Enzyme/Protease; NF-κB
Solubility:	H_2O : < 0.1 mg/mL;DMSO : 250 mg/mL (ultrasonic)



BIOLOGICAL ACTIVITY:

2-Methoxyestradiol (2-ME2), an orally active endogenous metabolite of 17β-estradiol (E2), is an **apoptosis** inducer and an **angiogenesis** inhibitor with potent antineoplastic activity. 2-Methoxyestradiol also destablize **microtubules**. 2-Methoxyestradio, also a potent superoxide dismutase **(SOD)** inhibitor and a ROS-generating agent, induces **autophagy** in the transformed cell line HEK293 and the cancer cell lines U87 and HeLa[1][2][3][4][5][6]. IC50 & Target:IC50: 1.2 μM (tubulin/microtubule, in living interphase MCF7 cells)[1] ***In Vitro:*** 2-Methoxyestradiol (2-ME) (5-100 μM) inhibits assembly of purified tubulin in a concentration-dependent manner, with maximal inhibition (60%) at 200 μM 2-Methoxyestradiol (2ME2). In living interphase MCF7 cells at the IC_{50} for mitotic arrest (1.2 μM), 2-Methoxyestradiol significantly suppresses the mean microtubule growth rate, duration and length, and the overall dynamicity, consistent with its effects in vitro, and without any observable depolymerization of microtubules. 2-Methoxyestradiol induces G_2-M arrest and apoptosis in many actively dividing cell types while sparing quiescent cells. 2-Methoxyestradiol binds to tubulin at or near the colchicine site, it inhibits microtubule assembly, and high concentrations have been shown to depolymerize microtubules in cells [1].

2-Methoxyestradiol (2-ME) decreases the HIF-1α and HIF-2α nuclear staining in cells cultured under hypoxia. 2-Methoxyestradiol is an anti-angiogenic, anti-proliferative and pro-apoptotic agent that suppresses HIF-1α protein levels and its transcriptional activity. A significant decrease in the growth rate is found in the 10 μM 2-Methoxyestradiol-treated A549 cells in comparison with the DMSO-treated cells (66.2±7.2 and 101.2±2.3%, respectively; p=0.04) at 96 h. A significant increase in apoptosis is observed in cells treated with 10 μM 2-Methoxyestradiol in a normoxic condition in comparison with cells under lower O_2 concentration (5.8±0.2%; p=0.003)[2]. ***In Vivo:*** To investigate the effect of 2-Methoxyestradiol (2-ME2) on uveitis development, C57BL/6 mice are randomly assigned into two groups and immunized with IRBP peptide. 2ME2 group starts 2-Methoxyestradiol (15 mg/kg) intraperitoneally from day 0 to day 13 while control group is given with vehicle. The disease score of 2-Methoxyestradiol (2ME2) group is 0.30±0.30, significantly lower than that of control group 2.09±0.28 (p<0.05), each group containing 5 mice[3].

Treatment with 2-Methoxyestradiol (60-600 mg/kg/d) results in a dose-dependent inhibition of tumor growth. The percentage of cells with strong pimonidazole-positive staining (+++) is significantly decreased in the 2-Methoxyestradiol-treated group (36.0% for 60 mg/kg/d and 0% for 200 and 600 mg/kg/d) compare with the vehicle-treated group (86.5%). This may be attributed to the dramatic inhibition of tumor growth in a dose-dependent manner following 2-Methoxyestradiol treatment[4].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [1]Microtubule protein (2.75 mg/mL) is assembled to steady-state [in 100 mM PIPES containing 1 mM EGTA and 1 mM $MgSO_4$ (PEM100) and 1 mM GTP, 35°C for 45 minutes] containing 2-Methoxyestradiol (final drug concentrations of 1-500 μM). Final DMSO and ethanol concentrations are adjusted to 1% and 5%, respectively. Concentrations of 2-Methoxyestradiol ≤ 5 μM have

no effect on microtubule polymer mass, and thus 20 to 500 μM 2-Methoxyestradiol is used for most of the experiments. Incubation with 2-Methoxyestradiol is carried out for 30 minutes, at which time microtubule depolymerization is maximal, and microtubules are centrifuged at 35°C for 30 minutes and the supernatant is removed from the pellets. Microtubule pellets are solubilized overnight in 0.2 M NaOH and the protein concentrations of supernatants and pellets are determined[1]. **Cell Assay:** 2-Methoxyestradiol (2ME2) is dissolved in DMSO (10 mM) and stored, and then diluted with appropriate media before use[1].[1]MCF7 breast carcinoma cells stably transfected with green fluorescent protein (GFP)-α-tubulin are cultured in DMEM supplemented with nonessential amino acids, 0.1% penicillin/streptomycin, 10% fetal bovine serum, and 0.4 mg/mL G418 at 37°C in 5% CO_2. Transfection of MCF7 cells with GFP-α-tubulin is carried out. To evaluate mitotic indices, cells are plated at a concentration of 6×10^4/2 mL into six-well plates. After 48 hours, cells are incubated in the absence or presence of 2-Methoxyestradiol at concentrations ranging from 100 nM to 30 μM for 20 hours. To collect both floating and attached cells, medium is collected; attached cells are rinsed with Versene (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, and 0.5 mM EDTA), detached by trypsinization, and added back to the medium. Cells are collected by centrifugation and fixed with 10% formalin for 30 minutes, permeabilized in ice-cold methanol for 10 minutes, and stained with 4′,6-diamidino-2-phenylindole to visualize nuclei. Results are the mean and SE of seven experiments in each of which 500 cells are counted for each concentration. The mitotic IC_{50} is the drug concentration that induced one half of the maximal mitotic accumulation[1]. **Animal Administration:** [3][4]Mice[3]

6~8-week-old C57BL/6 mice are used. C57BL/6 mice are immunized subcutaneously 0.1 mL at tail and 0.05 mL at both thigh sites with IRBP antigen complex. 500 ng Pertussis toxin is injected concurrently. This day is settled as day 0. Then mice are divided into 4 groups, each group containing 5 mice. 15 mg/kg 2-Methoxyestradiol or vehicle is abdominal injected during 0-13 days, 0-6 days, and 7-13 days. At day 14 eyes or lymphoglandula is collected after euthanasia.

Rats[4]

Fischer 344 rats (average body weight=150 g, n=6 per group) are treated with an i.p. injection of the vehicle (60, 200, or 600 mg/kg/d of 2-Methoxyestradiol/Panzem) for nine consecutive days beginning on the 8th day after the initial tumor cell injection. The experiment is repeated a second time using three rats per group.

References:

[1]. Kamath K, et al. 2-Methoxyestradiol suppresses microtubule dynamics and arrests mitosis without depolymerizing microtubules. Mol Cancer Ther. 2006 Sep;5(9):2225-33.

[2]. Aquino-Gálvez A, et al. Effects of 2-methoxyestradiol on apoptosis and HIF-1α and HIF-2α expression in lung cancer cells under normoxia and hypoxia. Oncol Rep. 2016 Jan;35(1):577-83.

[3]. Xu L, et al. 2-Methoxyestradiol Alleviates Experimental Autoimmune Uveitis by Inhibiting Lymphocytes Proliferation and T Cell Differentiation. Biomed Res Int. 2016;2016:7948345.

[4]. Kang SH, et al. Antitumor effect of 2-methoxyestradiol in a rat orthotopic brain tumor model. Cancer Res. 2006, 66(24),11991-11997.

[5]. LaVallee TM, et al. 2-Methoxyestradiol inhibits proliferation and induces apoptosis independently of estrogen receptorsalpha and beta. Cancer Res. 2002 Jul 1;62(13):3691-7.

[6]. Chen Y, et al. Oxidative stress induces autophagic cell death independent of apoptosis in transformed and cancer cells. Cell Death Differ. 2008;15(1):171-182.

CAIndexNames:

Estra-1,3,5(10)-triene-3,17-diol, 2-methoxy-, (17.beta.)-

SMILES:

OC1=CC2=C([C@]3(CC[C@@]4([C@H](CC[C@]4([C@@]3(CC2)[H])[H])O)C)[H])C=C1OC

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

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