# Ispinesib

**Cat. No.:** HY-50759

**CAS No.:** 336113-53-2

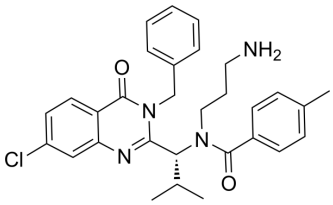
**Molecular Formula:** $C_{30}H_{33}ClN_4O_2$

**Molecular Weight:** 517.06

**Target:** Kinesin; Apoptosis

**Pathway:** Cell Cycle/DNA Damage; Cytoskeleton; Apoptosis

**Storage:** 4°C, protect from light
* In solvent : -80°C, 1 year; -20°C, 6 months (protect from light)



## SOLVENT & SOLUBILITY

**In Vitro**

DMSO : ≥ 125 mg/mL (241.75 mM)
* "≥" means soluble, but saturation unknown.

| Preparing Stock Solutions | Mass / Solvent / Concentration | 1 mg | 5 mg | 10 mg |
|---|---|---|---|---|
| | 1 mM | 1.9340 mL | 9.6701 mL | 19.3401 mL |
| | 5 mM | 0.3868 mL | 1.9340 mL | 3.8680 mL |
| | 10 mM | 0.1934 mL | 0.9670 mL | 1.9340 mL |

Please refer to the solubility information to select the appropriate solvent.

**In Vivo**

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (4.84 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (4.84 mM); Clear solution

## BIOLOGICAL ACTIVITY

**Description**

Ispinesib is a specific inhibitor of kinesin spindle protein (KSP), with a $K_{i\ app}$ of 1.7 nM.

**$IC_{50}$ & Target**

KSP
1.7 nM (Ki app)

**In Vitro**

Ispinesib is a potent, highly specific inhibitor of KSP, with a $K_{i\ app}$ of 1.7 nM[1].
Ispinesib (150 nM) inhibits BT-474 and MDA-MB-468 cell lines, with $GI_{50}$s of 45 and 19 nM, respectively[2].
Ispinesib (SB715992, 15 and 30 nM) suppresses the proliferation of PC-3 prostate cancer cell by 48.65% and 52.16%, and induces apoptosis of prostate cancer cell by 1094.88% and 1516.70%, respectively. Ispinesib up regulates genes responsible for apoptosis and cell cycle arrest, and down regulates genes responsible for cell proliferation and survival. The anti-

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|---|---|
| | proliferation and pro-apoptotic activities of Ispinesib can be enhanced by genistein[3].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |
| **In Vivo** | Ispinesib (SCID, 8 mg/kg; nude, 10 mg/kg, q4d × 3) reduces tumor volume in mice bearing tumor xenografts of ER-positive (MCF7), HER2-positive (KPL4, HCC1954, and BT-474), and triple-negative (MDA-MB-468) breast cancer cells via i.p. one dose every 4 days repeated three times[2].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |

## PROTOCOL

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| **Kinase Assay** [1] | Kinesin specificity analysis is carried out using a pyruvate kinase–lactate dehydrogenase detection system that couples the production of ADP to oxidation of NADH. Absorbance changes are monitored at 340 nm. Steady-state studies using nanomolar concentrations of KSP are performed using a sensitive fluorescence-based assay utilizing a pyruvate kinase, pyruvate oxidase, and horseradish peroxidase coupled detection system that couples the generation of ADP to oxidation of Amplex Red to fluorescent resorufin. Generation of resorufin is monitored by fluorescence (λexcitation = 520 nm and λ emission= 580 nm). Steady-state biochemical experiments are performed in PEM25 buffer [25 mM Pipes-$K^+$ (pH 6.8), 2 mM $MgCl_2$, 1 mM EGTA] supplemented with 10 μM paclitaxel for experiments involving microtubules. The $IC_{50}$ for steady-state inhibition is determined at 500 μM ATP, 5 μM MTs, and 1 nM KSP in PEM25 buffer. $K_{i\ app}$ (apparent inhibitor dissociation constant) estimates of Ispinesib are extracted from the concentration-response curves, with explicit correction for enzyme concentration[1].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |
| **Cell Assay** [3] | PC-3 prostate cancer cells are seeded in 96 well plates at a density of $4 \times 10^3$ cells/well. PC-3 cells are incubated for 24 hours to allow attachment to the surface of each well of the tissue culture plate. Then, the cells are treated with varying concentration of reagents and incubated for 1 to 3 days. First, PC-3 cells are treated with 15 and 30 nM of Ispinesib, respectively. Second, PC-3 cells are subjected to combinational treatments with 7.5 or 10 nM of Ispinesib plus 30 μM of genistein. Finally, PC-3 cells are pre-treated with 30 μM of genistein for 24 hours followed by treatment with 15 nM of Ispinesib. Control cells are treated with 0.3 mM $Na_2CO_3$ (vehicle control). After treatment, PC3 cells are incubated at 37°C with MTT (0.5 mg/mL) for 2 hours and isopropyl alcohol at room temperature for 1 hour. The spectrophotometric absorbance of each sample is then determined by using ULTRA Multifunctional Micro Plate Reader at 595 nm[3].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |
| **Animal Administration** [2] | Mice with a tumor volume of -250 $mm^3$ receive a single dose of Ispinesib (10 mg/kg). Tumors are dissected, fixed in 10% buffered formalin, and embedded in paraffin, and 5-μm tissue sections are prepared. Antigen retrieval is done by boiling in 50 mM citrate buffer (pH 5.5), and sections are then incubated in 3% hydrogen peroxide, washed in PBS-0.1% Tween, and blocked in 10% goat serum. Phospho-histone H3 (PH3) antibody is detected using Alexa Fluor 488 secondary antibody. Images are taken with a microscope at ×10 magnification and captured using MetaMorph software to quantify PH3 expression by computing the area ratio of PH3-positive cells per total cells. Ki67/cleaved caspase-3 staining is done. Nonfluorescent images are taken on an Olympus BX41 microscope at ×20 magnification[2].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |

## CUSTOMER VALIDATION

- Redox Biol. 2019 Feb;21:101112.
- Cancer Lett. 2021 Feb 27.
- Urol Oncol. 2023 Feb 20;S1078-1439(23)00010-8.
- Preprints. 2023 Sep 30.
- bioRxiv. 2023 Sep 12.

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## REFERENCES

[1]. Lad L, et al. Mechanism of inhibition of human KSP by ispinesib. Biochemistry. 2008 Mar 18;47(11):3576-85.

[2]. Purcell JW, et al. Activity of the kinesin spindle protein inhibitor ispinesib (SB-715992) in models of breast cancer. Clin Cancer Res. 2010 Jan 15;16(2):566-76.

[3]. Davis DA, et al. Increased therapeutic potential of an experimental anti-mitotic inhibitor SB715992 by genistein in PC-3 human prostate cancer cell line. BMC Cancer. 2006 Jan 24;6:22.



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