**Proteins** 

# Inhibitors

### Ethoxyquin

Cat. No.: HY-B1425 CAS No.: 91-53-2 Molecular Formula: C<sub>14</sub>H<sub>19</sub>NO Molecular Weight: 217.31

Target: HSP; Reactive Oxygen Species

Pathway: Cell Cycle/DNA Damage; Metabolic Enzyme/Protease; Immunology/Inflammation;

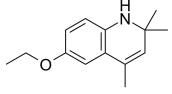
NF-κB

Storage: Pure form -20°C 3 years

> 4°C 2 years

In solvent -80°C 6 months

-20°C 1 month



**Product** Data Sheet

#### **SOLVENT & SOLUBILITY**

In Vitro

DMSO:  $\geq 50 \text{ mg/mL} (230.09 \text{ mM})$ 

H<sub>2</sub>O: < 0.1 mg/mL (insoluble)

\* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent Mass Concentration	1 mg	5 mg	10 mg
	1 mM	4.6017 mL	23.0086 mL	46.0172 mL
	5 mM	0.9203 mL	4.6017 mL	9.2034 mL
	10 mM	0.4602 mL	2.3009 mL	4.6017 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: ≥ 3.5 mg/mL (16.11 mM); Clear solution
- 2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 3.5 mg/mL (16.11 mM); Clear solution
- 3. Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 3.5 mg/mL (16.11 mM); Clear solution

#### **BIOLOGICAL ACTIVITY**

Description Ethoxyquin is an antioxidant which has been used in animal feed for many years and also an inhibitor of heat shock protein

90 (Hsp90).

IC<sub>50</sub> & Target HSP90

#### In Vitro

Ethoxyquin (EQ) does not block Paclitaxel's (PTX's) ability to induce cell death in 4 different breast cancer cell lines. Results demonstrate that only when Hsp90 level is down regulated is neuroprotection provided by Ethoxyquin (EQ) no longer seen  $^{[2]}$ . It is found that Ethoxyquin provides neuroprotection with a best efficacy range from 30 to 300 nM. It is interesting to note that higher ( $\mu$ M) concentrations of Ethoxyquin do not provide further neuroprotection and in fact at these higher concentrations Ethoxyquin loses its neuroprotective effects  $^{[3]}$ .

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

#### In Vivo

Ethoxyquin (EQ) provides a dose-dependent neuroprotection. Although all 3 doses of Ethoxyquin provide partial neuroprotection against reduction in intraepidermal nerve fiber density, the peak efficacy is with the 75  $\mu$ g/kg dose<sup>[2]</sup>. Rats treated with Ethoxyquin (EQ) alone or control vehicle treated rats gain weight as expected under normal physiological conditions<sup>[3]</sup>.

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#### **PROTOCOL**

#### Kinase Assay [2]

To evaluate the impact of Ethoxyquin (EQ) on Paclitaxel's (PTX's) ability to kill breast cancer cells, conditions for culturing 4 cancer cell lines and measuring the ATP levels are optimized for the 96-well plate format. Briefly, 1,500 cells/well in media are plated in 96-well plates for 24 hours. Constant concentrations of PTX with or without Ethoxyquin are added to the wells for another 24 hours. Cellular ATP levels are measured using kit according tomanufacturer's protocol<sup>[2]</sup>.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

#### Cell Assay [2]

Briefly, DRGs are harvested from embryonic day 14.5 rats according to standard protocols, then cells are plated onto collagen-coated glass coverslips and allowed to extend neurites for 24 hours in media (Neurobasal medium, 50 mM pencillin-streptomycin (PS), 0.2% FBS, 0.5 mM glutamine, 1×B-27 supplement, 0.2% glucose,10 ng/mL glial cell line-derived neurotrophic factor). Ethoxyquin (EQ) or vehicle control is added to the wells for another 24-hour incubation. DRG cells are fixed with 4% paraformaldehyde and stained with anti-βIII-tubulin antibody to delineate the axons. Axon lengths are measured in multiple fields using a random sampling method<sup>[2]</sup>.

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## Animal Administration [2]

To examine the effect of Ethoxyquin (EQ) on Paclitaxel's (PTX's) ability to reduce tumor burden in vivo, a mouse model in which breast cancer cell line SUM-159 (3×10<sup>6</sup> tumor cells suspended in phosphate-buffered saline in a final volume of 0.15 mL) is injected subcutaneously into adult male nude mice. When the tumor size reaches 5 mm in diameter, the animals are randomly assigned to PTX or PTX with Ethoxyquin groups. Ethoxyquin is given by intraperitoneal administration on a daily basis for 3 weeks. At the end of 3 weeks, animals are euthanized and tumor size and weight are measured (n=5 per group)<sup>[2]</sup>. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

#### **REFERENCES**

[1]. Sadikot T, et al. Development of a high-throughput screening cancer cell-based luciferase refolding assay for identifying Hsp90 inhibitors. Assay Drug Dev Technol. 2013 Oct;11(8):478-88.

[2]. Zhu J, et al. Ethoxyquin prevents chemotherapy-induced neurotoxicity via Hsp90 modulation. Ann Neurol. 2013 Dec;74(6):893-904.

[3]. Zhu J, et al. Ethoxyquin provides neuroprotection against cisplatin-induced neurotoxicity. Sci Rep. 2016 Jun 28;6:28861.

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

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