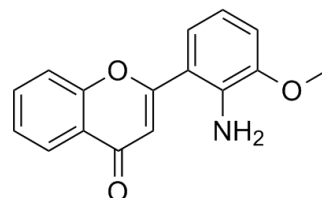


## Data Sheet

Product Name:	PD98059
Cat. No.:	HY-12028
CAS No.:	167869-21-8
Molecular Formula:	C <sub>16</sub> H <sub>13</sub> NO <sub>3</sub>
Molecular Weight:	267.28
Target:	Autophagy; MEK
Pathway:	Autophagy; MAPK/ERK Pathway
Solubility:	DMSO: 16 mg/mL; H <sub>2</sub> O: < 0.01 mg/mL



### BIOLOGICAL ACTIVITY:

PD98059 is a **MEK** inhibitor with **IC<sub>50</sub>** of 5  $\mu$ M, also suppresses TCDD binding to the aryl hydrocarbon receptor (AHR) with **IC<sub>50</sub>** of 4  $\mu$ M. IC<sub>50</sub> & Target: IC<sub>50</sub>: 5  $\mu$ M (MEK)<sup>[1]</sup>

**In Vitro:** Concentrations of PD98059 of  $\leq 20$   $\mu$ M are not cytotoxic to cultured MCF10A, MCF10A-Neo, and MCF10A-NeoT cells. However, PD98059 is weakly cytostatic to all three lines at concentrations of  $\geq 10$   $\mu$ M. Treatment of MCF10A-Neo and MCF10A-NeoT cultures with concentrations of PD98059 up to 20  $\mu$ M for 2-22 hr does not alter the total ERK content. However, treatment with PD98059 does result in concentration-dependent reductions in the dually phosphorylated forms of ERK1 and ERK2. Within 2 hr of a 10- $\mu$ M treatment, phosphorylated ERK contents are reduced  $\sim 74\%$  and  $\sim 86\%$  in MCF10A-Neo and MCF10A-NeoT cultures, respectively (IC<sub>50</sub>=1  $\mu$ M). Within 22 hr of treatment, phosphorylated ERK forms are almost completely eliminated in both cell lines<sup>[1]</sup>. PD98059 (PD 098059) prevents the activation of MAPKK1 by Raf or MEK kinase in vitro at concentrations (IC<sub>50</sub>=2-7  $\mu$ M). PD98059 inhibits both the activation and phosphorylation of MAPKK1 in vitro by either c-Raf or MEK kinase with IC<sub>50</sub> values of 4 $\pm$ 2  $\mu$ M. Incubation of Swiss 3T3 cells with PD98059 (50  $\mu$ M) suppressed by 80-90% the activation of MAPKK induced by each agonist, but the activation of c-Raf is enhanced 2-3-fold<sup>[2]</sup>.

**In Vivo:** The treatment of mice with PD98059 significantly reduces the level of p-ERK1/2. Moreover, a significant increase in the phospho-p38 expression is observed in Zymosan-treated mice at 18 h after Zymosan administration compared to the sham-operated mice. The treatment with PD98059 significantly reduces the p38 expression<sup>[3]</sup>. Repeated treatment with PD98059 attenuates mechanical allodynia measured by the von Frey test three (18.0 g $\pm$ 0.8, n=10) and seven (20.21 g $\pm$ 0.67, n=26) days after CCI in comparison to the vehicle-treated CCI-exposed rats (15.1 g $\pm$ 1.3, n=7 and 14.21 g $\pm$ 0.44, n=28, respectively). Repeated injection of PD98059 diminishes thermal hyperalgesia, as is evaluated by the cold plate test, three (17.5 s $\pm$ 2.1, n=10) and seven (25.54 s $\pm$ 1.03, n=26) days following CCI compared to vehicle-treated CCI-exposed rats (11.5 s $\pm$ 1.8, n=7 and 11.4 s $\pm$ 0.88, n=28, respectively)<sup>[4]</sup>.

### PROTOCOL (Extracted from published papers and Only for reference)

**Kinase Assay:** <sup>[1]</sup>Kinase reactions are performed in 50  $\mu$ L reaction volumes and contain 50 mM Tris, pH 7.4, 10 mM MgCl<sub>2</sub>, 2 mM EGTA, 10  $\mu$ M ATP (containing 1  $\mu$ Ci of 3000 Ci/mmol [ $\gamma$ -<sup>32</sup>P]ATP), 7.6  $\mu$ g of GST-MEK1, 7.2  $\mu$ g of GST-ERK1, and 20  $\mu$ g of MBP. PD98059 and other flavonoids are added to the reactions mixtures immediately after the addition of GST-MEK1 but before the addition of GST-ERK1 and ATP. Control reactions contain ERK1 and MBP but no MEK. Reaction mixtures are incubated at 30°C for 15 min before being stopped by the addition of Laemmli's SDS sample buffer. Proteins are separated on SDS-15% polyacrylamide gels. After vacuum drying of the gel, radioactivity is detected by autoradiography on X-ray film or phosphoimaging using a BioRad GS-525 Molecular Imager<sup>[1]</sup>. **Cell Assay:** PD98059 is dissolved in DMSO and stored, and then diluted with appropriate media (DMSO <0.1%) before use<sup>[1]</sup>.

<sup>[1]</sup>The MCF10A, MCF10A-Neo, and MCF10A-NeoT cell lines are used. Subconfluent cultures are treated with PD98059 (0-100  $\mu$ M). Viability of cells after treatment is assessed by ability to exclude trypan blue. Cultures earmarked for RNA isolation are washed twice with phosphate-buffered saline (2.7 mM KCl, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, 137 mM NaCl, 8 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.2) at harvesting and stored at -80°C

[1]. **Animal Administration:** PD98059 is prepared in non-pyrogenic saline (0.9% NaCl) (Mice)<sup>[3]</sup>.

PD98059 is dissolved in 75% DMSO (Rat)<sup>[4]</sup>.<sup>[3]</sup><sup>[4]</sup>Mice<sup>[3]</sup>

Male CD mice (20-22 g) are randomly allocated into the following groups: 1. Zymosan+DMSO group. Mice are treated intraperitoneally (i.p.) with Zymosan (500 mg/kg, suspended in saline solution) and with the vehicle for PD98059 (10% DMSO, v/v) i.p. 1 and 6 h after Zymosan administration (N=10). 2. PD98059 group. Identical to the Zymosan+DMSO group but are administered PD98059 (10 mg/kg, i.p. bolus) at 1 and 6 h after Zymosan (N=10) instead of DMSO. 3. Sham+DMSO group. Identical to the Zymosan+DMSO group but are administered saline solution instead of Zymosan (N=10). 4. Sham+PD98059 group. Identical to Sham+DMSO group, except for the administration of PD98059 (10 mg/kg i.p. bolus) 1 and 6 h after saline administration (N=10).  
Rat<sup>[4]</sup>

The rats (male Wistar, 300-350 g) are used. The PD98059 (2.5 µg/5 µL, i.t.) is single or repeated preemptively administered 16 h and 1 h before CCI and then once daily for 7 days. The Vehicle-treated CCI-exposed rats receive 75% DMSO according to the same schedule. There is no significant difference in pain behavior between no-treated and V(DMSO)-treated CCI-exposed rats. This method of PD98059 or vehicle administration is used throughout the study and is referred to in the text as "repeated administration". At day 7<sup>th</sup> after CCI 30 min after PD98059 administration tactile allodynia is measured using von Frey test and thermal hyperalgesia is conducted using cold plate test. Additionally, at day 7<sup>th</sup> after CCI the vehicle-treated and PD98059-treated rats receive a single i.t. vehicle, Morphine (2.5 µg/5 µL) or Buprenorphine (2.5 µg/5 µL) injection 30 min after PD98059, and then 30 min later the von Frey and/or cold plate tests are repeated. Since the dose of morphine 2.5 µg/5 µL in naive rats produces maximal analgesic effect in tail-flick test. Lower dose of Morphine are used for co-administration experiments, so that observing the possible enhancement of opioid effectiveness. The vehicle-treated and PD98059-treated naive rats (uninjured rats) receive a single i.t. vehicle, Morphine (0.5 µg/5 µL) or Buprenorphine (2.5 µg/5 µL) injection 30 min after PD98059, and then 30 min later the tail flick test is performed.

## References:

- [1]. Reiners JJ Jr, et al. PD98059 is an equipotent antagonist of the aryl hydrocarbon receptor and inhibitor of mitogen-activated protein kinase kinase. *Mol Pharmacol.* 1998 Mar;53(3):438-45.
- [2]. Alessi DR, et al. PD 098059 is a specific inhibitor of the activation of mitogen-activated protein kinase kinase in vitro and in vivo. *J Biol Chem*, 1995, 270(46), 27489-27494.
- [3]. Di Paola R, et al. PD98059, a specific MAP kinase inhibitor, attenuates multiple organ dysfunction syndrome/failure (MODS) induced by zymosan in mice. *Pharmacol Res.* 2010 Feb;61(2):175-87.
- [4]. Rojewska E, et al. PD98059 Influences Immune Factors and Enhances Opioid Analgesia in Model of Neuropathy. *PLoS One.* 2015 Oct 1;10(10):e0138583.

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

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