VPR-66, ROR gamma T and ROR alpha Inhibitor

Catalog No:	**NBP2-29362**
Storage:	As a solid powder, the inhibitor is stable in the desiccator at -20°C for 1 year. DMSO-reconstituted solution is stable for up to 2 month at -20°C.
Form:	Solid Powder; molecular weight: 450.61

Background

Celastrol is a pentacyclic-triterpene extracted from Tripterygium wilfordii (Thunder of God Vine). This IL-17A inhibitor is known for its role in the prevention of inflammatory diseases and cancer (1). Molecular targets include inhibition of IKKa/b kinases, inactivation of Cdc37 and p23 proteins that are co-chaperones of HSP90, inhibition of proteasome function, and activation of heat-shock transcription factor 1 (HSF1) (2). A recent study showed that this inhibitor suppressed rheumatoid arthritis through modulation of the key proinflammatory cytokines such as IL-17, IL-6 and IFNg; the authors indeed observed that those cytokine responses were significantly inhibited in arthritic rats when treated with the inhibitor (3). Our data also show that it directly suppresses IL-17A induction, in which constitutive activation of the IL-17A promoter in the IL-17A Prom/LUCPorter™ HEK 293 cell line (Novus Biologicals, NBP2-26283) has been highly inhibited by celastrol with the IC50 of 0.1545 μM (Figure 1). Furthermore, the inhibitor suppresses IL-17A production in peripheral blood mononuclear cells stimulated with anti-CD3/CD28 (Figure 2).

Preparation

Note: Please read the entire data sheet before using this product.

1. To make a 50 mM stock solution, dissolve 1 mg celastrol in 44.5 ul anhydrous dimethyl sulfoxide (DMSO) by gentle vortex.
2. Divide into useable aliquots and store them at -20oC.
3. The stock solution may be diluted further to make working solutions in DMSO. The final DMSO concentration in the cells to be analyzed should not exceed 1%.

Usage:

Celastrol is used in assays to inhibit IL-17A induction. We recommend an initial titration of the inhibitor from 0-50 uM for in vitro assays along with vehicle. The IL-17A Prom/LUCPorter™ cell line (NBP2-26283), which is a semi-constitutively active cell line, is a useful positive control model system for studying inhibition of IL-17A induction by celastrol (Figure 1). The anti-CD3/CD28-stimulated peripheral blood mononuclear cells (PBMC) are another model system for studying inhibition of IL-17A production (Figure 2).

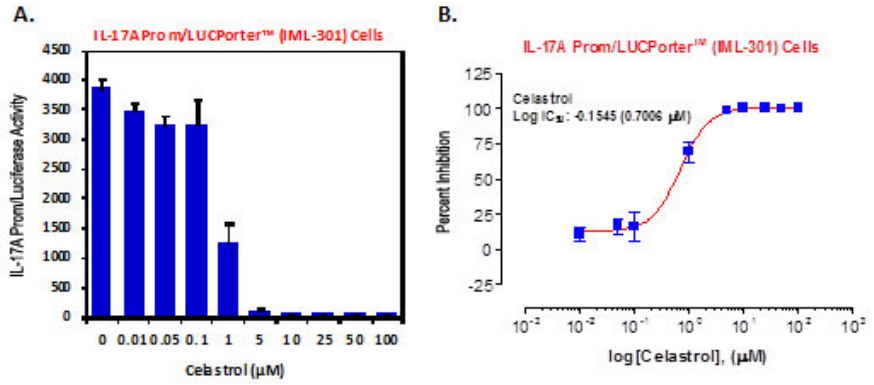

Figure 1. Evaluation of inhibitory activity on induction of IL-17A. IL-17A Prom/ LUCPorterTM HEK 293 (IML-301) cells were plated in 96-well white plates at 5 x 10^4 cells/well for 16 h. Cells were treated with different concentrations of IL-17A inhibitor between 0 and 100 µM as noted [A] for 6 h. The luciferase reporter assay reagent (LS010) was then directly added to the cell plates (50 µl/well). After 10 min, the plates were analyzed by reading in a plate luminometer. The values from [A] were used to determine the IC50 of the IL-17A inhibitor [B].

Data Summary: Celastrol inhibited the IL-17A promoter induction in a dose-response manner, of which IC50 was measured as 0.7 uM.

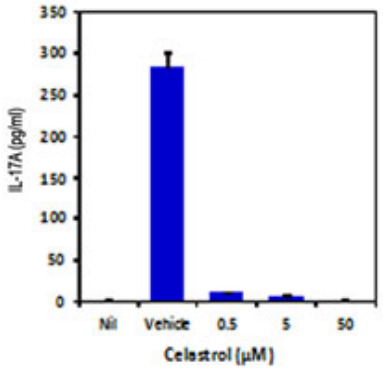

Figure 2. The inhibitor suppresses IL-17A production in human PBMC stimulated with anti-CD3/CD28. Perpheral blood mononuclear cells (PBMC) were stimulated with anti-CD3 and anti-CD28 in the presence or absence of celastrol (0.5, 5 and 50 µM) for 3 days. IL-17A was then measured from the cell culture media using the Human IL-17A ActivELISA™ (IMK-540XL-2).

Data Summary: PBMCs that were stimulated with anti-CD3/CD28 produced IL-17A, of which induction was inhibited by the IL-17A inhibitor. Vehicle: DMSO, Nil: no anti-CD3/CD28 stimulated PBMCs.

Reference:

1. Gupta, S. C. et al. (2010). Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. Cancer Metastasis Rev. 29, 405-434.
2. Salminen, A. et al. (2010). Celastrol: Molecular targets of Thunder God Vine. *Biochem Biophys Res Commun*.394, 439-442.
3. Venkatesha, S. H. et al. (2011). Celastrus-derived celastrol suppresses autoimmune arthritis by modulating antigen-induced cellular and humoral effector responses. *J. Biol. Chem.* 286, 15138-15146.