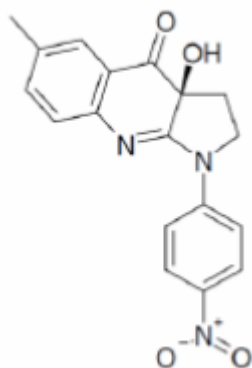


## Para-nitroblebbistatin

Cat. No. ax494693



## Product Specification

(S)-3a-Hydroxy-6-methyl-1-(4-nitro-phenyl)-1,2,3,3a-tetrahydro-pyrrolo[2,3-b]quinolin-4-one

Parameter	Results
M.w	337.34
Storage	-20
Chemical purity	>95%
Ratio of enantiomer	>90%
Cytotoxicity	Not cytotoxic in the dark
Extinction coeff at 427 nm (pH 7.3)	11000 M <sup>-1</sup> cm <sup>-1</sup>
Extinction coeff at 430 nm (in 100% DMSO)	15000 M <sup>-1</sup> cm <sup>-1</sup>

## Recommendations For Use

1. Solubility: When using para-nitroblebbistatin (pNB) in water-based buffers, always apply fresh dilution from DMSO stock, otherwise the effective concentration may significantly decrease (Fig 2) similarly to blebbistatin (B). Once pNB or B is added to cells, the effective concentration will not change anymore, allowing treatments for longer time periods. Use maximum 20µM concentration to avoid aspecific effects.

2. Photostability: Based on tests with HeLa cells unlike B, pNB is not phototoxic when irradiated with >440 nm wavelength light. However, upon <400 nm irradiation the two compounds are equally phototoxic.

3. Freeze thaw: limit freeze-thawing cycles.

## Data Figures

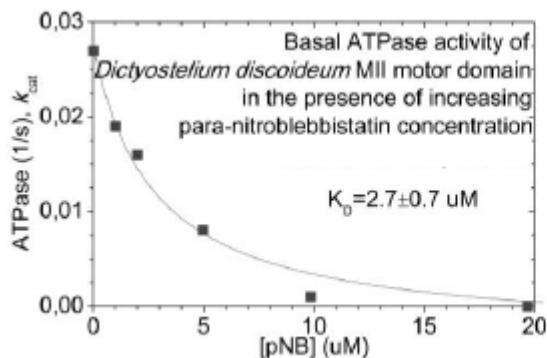


Figure 1 Affinity and inhibitory properties of para-nitroblebbistatin (pNB) for *Dictyostelium discoideum* myosin 2.

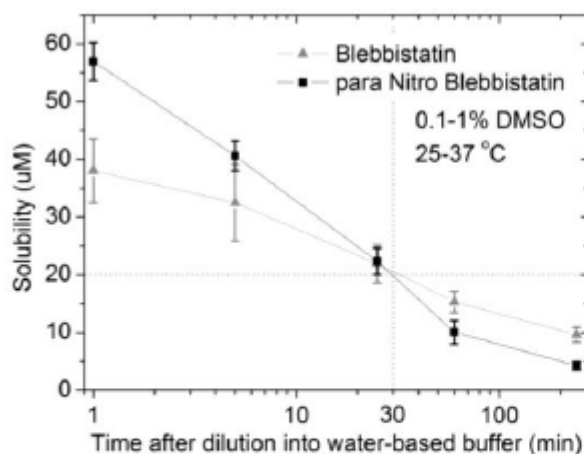


Figure 2 Solubility of para-nitroblebbistatin (pNB) and blebbistatin (B). B and pNB DMSO stock solutions were diluted into water-based buffer (20mM HEPES, pH 7.3, 40mM NaCl, 4mM MgCl<sub>2</sub>) to a nominal concentration of 60 uM. The solutions were centrifuged immediately (at 1 min) and after 5, 25, 60, 240 minutes. From the absorbance of the supernatant at 427 nm, the effective

concentrations of the compounds were determined, which are plotted on the graph. Temperature between 25-37 °C and DMSO concentration within the 0.1-1% range had practically no effect on solubility.

## Usage Statement

Our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans.

## Ethics Statement

Primary cell and primary cell-derived products sold by Axol Bioscience are obtained with fully informed consent including consent for commercial use from the donor or donor's next-of-kin. An independent Ethics Committee or Institutional Review Board approved the donor consent forms. The anonymized cells/tissue are collected under protocols that are in compliance with the Health Insurance Portability and Accountability Act of 1996 (HIPAA), the Declaration of Helsinki or Human Tissue Act, as appropriate. The collection protocol of sample material must comply with all international, national and local laws. Axol Bioscience works through tissue acquisition networks, physicians and organizations that are compliant with these standards.

## QC Review

<b>Reviewer</b>	<b>Position</b>	<b>Signature</b>	<b>Date</b>
Rob Treanor	Head of Supply		May 2018