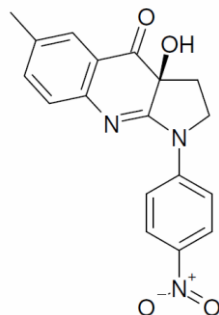


Name of chemical: para-nitroblebbistatin

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

Cat. No: DR-N-111



M.w: 337.34

Extinction coeff at 427 nm (pH 7.3): 11000 M⁻¹cm⁻¹

Extinction coeff at 430 nm (in 100% DMSO): 15000 M⁻¹cm⁻¹

Chemical purity: >95%

Ratio of (-) enantiomer: >90%

Storage conditions: in powder form or dissolved in 100% DMSO in aliquots at -20 °C to limit freezing-thawing cycles

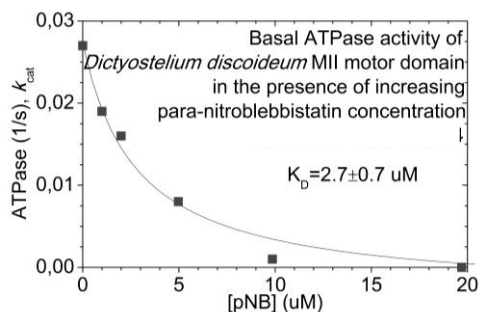


Figure 1 Affinity and inhibitory properties of para-nitroblebbistatin (pNB) for Dictyostelium discoideum myosin 2.

Reference: **Para-nitroblebbistatin, the non-cytotoxic and photostable myosin II inhibitor** by M. Képiró, B. H. Várkuti, L. Végner, G. Vörös, Gy. Hegyi, M. Varga, A. Málnási-Csizmadia in *Angewandte Chemie* Volume 53, Issue 31, July 28, 2014, DOI: 10.1002/anie.201403540

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.

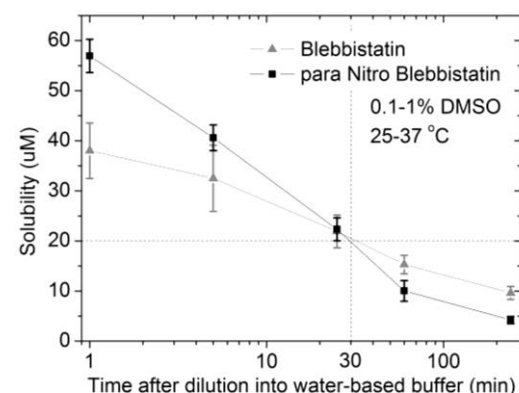


Figure 2 **Solubility of para-nitroblebbistatin (pNB) and blebbistatin (B)**. B and pNB DMSO stock solutions were diluted into water-based buffer (20mM HEPES, pH 7.3, 40mM NaCl, 4mM MgCl₂) to a nominal concentration of 60 uM. The solutions were centrifuged immediately (at 1 min) and after 5, 25, 60, 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.

2. Use maximum 20 uM concentration to avoid aspecific effects.
3. 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.
4. Cytotoxicity in dark: Unlike B, pNB is not cytotoxic in the dark. This was tested on HeLa cells, where mortality was identical to control after 3 days of 20 uM pNB treatment.