

Gatastatin G2 < γ -Tubulin Inhibitor>

Catalog NO. FDV-0040

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Product Background

The tubulin superfamily is one of the GTPases known major components of microtubules (MTs) and in humans, has five sub-members, α -, β -, γ -, δ , and ϵ -tubulin. Among the member of the tubulin family, α -, β - and γ -tubulins are ubiquitously expressed. α - and β -tubulins form heterodimers and the α/β -tubulin dimers polymerized to MTs. On the other hand, γ -tubulin is not incorporated into MTs directly but it interacts with 5 proteins to form γ -tubulin ring complex (γ TuRC). γ TuRC plays an important role in the initiation of MT polymerization from centrosome (Figure 1). γ TuRC is observed not only around centrosomes but also in branch sites of MTs. Many researchers indicate γ -tubulin in γ TuRC an essential component to form and regulate MTs in cells. However, the molecular mechanism of γ -tubulin-based MT initiation and regulation is still unclear. Furthermore, recent studies suggest γ -tubulin is not limited to γ TuRC, but there are additional filament structures containing self-assembled γ -tubulins such as γ -string, γ -tubules, etc. These γ -tubulins associate with cell organelles and may support organelle structures. Moreover, γ -tubulin binds to the DNA on the same DNA binding motif of E2F (E2 promoter-binding factor), a transcription factor, and is considered as a competitive regulator of E2F for gene expression. Although γ -tubulin plays various roles in cellular processes, analytical methods for the γ -tubulin function are still limited. RNA interference (RNAi) is the major approach to investigate γ -tubulin functions so far. Because RNAi requires over a half day to reduce protein γ -tubulin expression, growing cells are preferentially stopped on mitosis and induced apoptosis due to lack of γ TuRC for MT initiation from centrosomes. As γ TuRC is an essential component in cells, RNAi strategy induces cell death preferentially and insufficient method to monitor various γ -tubulin functions excepting γ TuRC. To approach γ -tubulin functions, small compound inhibitors are desired because inhibitors can block γ -tubulin function at any time. However, no inhibitor which has little effects on α - and β -tubulins and blocks γ -tubulin specifically has been discovered so far.

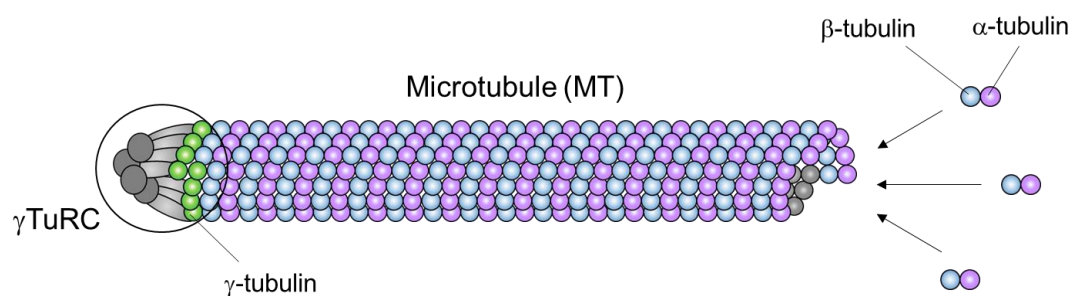


Figure 1. Overview of γ -tubulin function on MT formation

Gatastatin was originally discovered in 2015 by Dr. Elmar Schiebel, University of Heidelberg, Dr. Takeo Usui, University of Tsukuba, and Dr. Ichiro Hayakawa, Nihon University, as the world's first γ -tubulin-specific inhibitor. Gatastatin is a derivative of Glaziopianin A, a natural compound derived from a bean, and blocks GTP-binding to the γ -tubulin protein. In 2020, Dr. Usui group further succeeded in the identification of a superior derivative of Gatastatin, called 2nd generation Gatastatin (**Gatastatin G2**). Gatastatin G2 shows about ten higher inhibition activity than prototype-Gatastatin in cell-based experiments. Gatastatin G2 has little effects on α/β -tubulin polymerization both *in vitro* and in cells. Ref.1 showed Gatastatin G2 induced abnormal chromosome alignment and multipolar formation in mitotic cells.

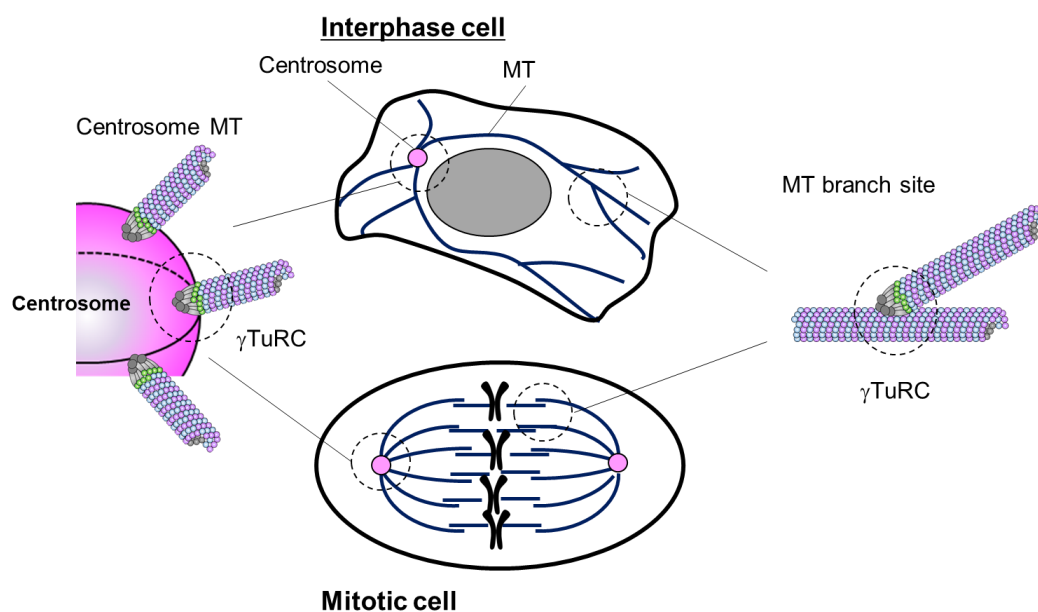
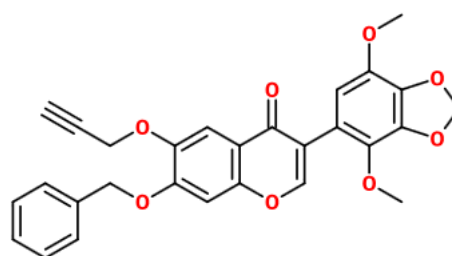


Figure 2. Overview of γ -tubulin function in interphase cell and mitotic cells

Description

Catalog Number: FDV-0040
 Size: 0.1 mg
 Formulation: C₂₈H₂₂O₈
 Molecular weight: 486.47 g/mol
 Structure: See right figure
 Solubility: Soluble in DMSO



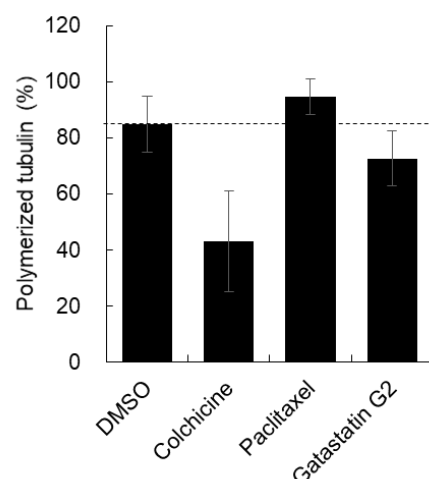
Reconstitution and Storage

Reconstitution: Stock solution recommended concentration 1 mM in 100% DMSO.
 Storage (powder): Store powder at -20°C.
 Storage (solution): After reconstitution in DMSO, aliquot and store at -20 °C.
 Avoid repeated freeze-thaw cycles.

Reference data

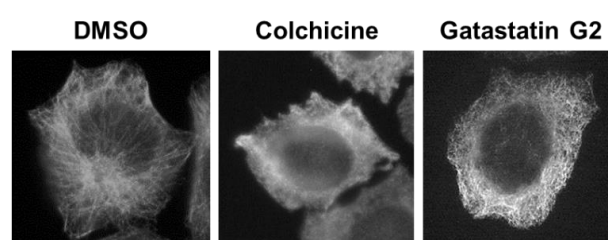
Effect of Gatastatin G2 on MT polymerization *in vitro*

Purified tubulin (1 mg/ml) from porcine brain was polymerized by 0.8 M glutamate for 30 min at 37°C with or without 10 μ M colchicine (an inhibitor of MT), 10 μ M paclitaxel (an activator of MT polymerization) and 30 μ M Gatastatin G2. Polymerized and unpolymerized tubulins were separated by ultracentrifugation. Polymerization ratio (%) was estimated by SDS-PAGE. Colchicine clearly inhibited MT polymerization, paclitaxel promoted MT polymerization but Gatastatin G2 showed little effect on MT polymerization.



Effect of Gatastatin G2 on MT structure in cells

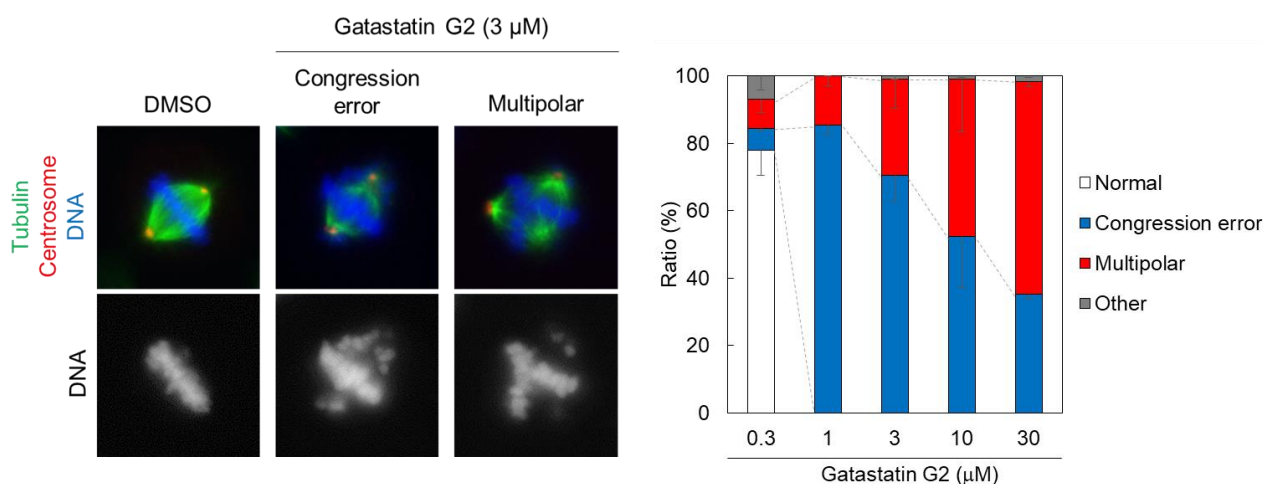
HeLa cells were treated with 100 nM colchicine for 6 hours or 30 μ M Gatastatin G2 for 24 hours. After chemical treatment, the cells were fixed with ice-cold MeOH and visualized by anti- α -tubulin antibody and fluorophore-conjugate secondary antibody. While colchicine induced abnormal MT structure, Gatastatin G2 showed little effect on cellular MT structure.



Application data

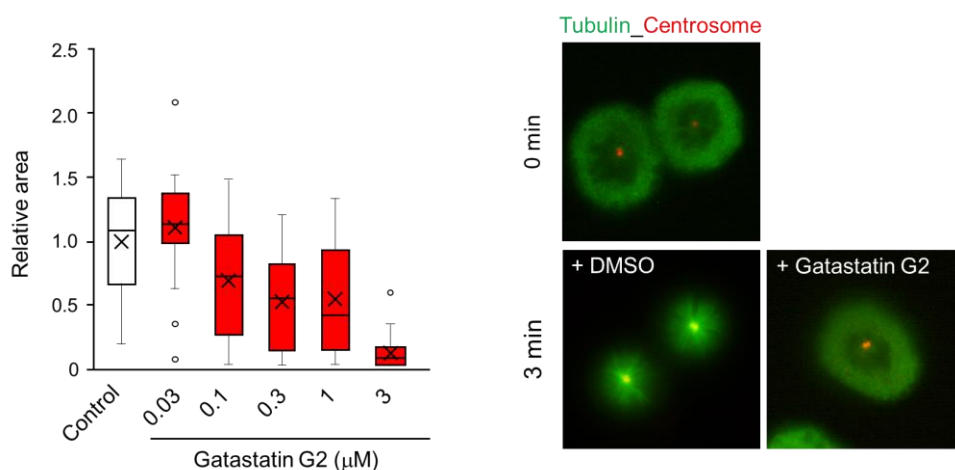
Gatastatin G2 induced abnormal spindle formation in mitotic cells

Exponentially growing HeLa cells were treated with 0.3-30 μ M Gatastatin G2 for 24 hours and were fixed and stained with anti- α -tubulin for spindle fibers, anti-pericentrin for centrosome and DAPI for chromosomal DNAs. The resulting spindle morphology was classified and quantified. At the lower concentration of Gatastatin G2, misaligned chromosomes were mainly observed. On the other hand, high concentration often induced multipolar spindle formation rather than congression error. Multipolar induction was clearly dose-dependently observed. This experiment suggests γ -tubulin regulates both chromosome movement and normal bipolar formation in mitotic cells.



Gatastatin G2 blocks centrosome-derived MT formation in mitotic cells

HeLa cells (6.0×10^3 cells) were seeded, cultured for 1 hour, and subsequently treated with *S*-trityl-*L*-cysteine (STLC; $20 \mu\text{M}$) for 6 hours. Then, the cells were washed with ice-cold medium and incubated on ice to depolymerized MT for 1 hour. The cells were treated with 1% DMSO, or 0.03 - $3 \mu\text{M}$ Gatastatin G2 for 15 min on ice. During the process of drug treatment on ice, control cells were fixed with MeOH (as 0 min in the figure). After drug treatment, the cell media was exchanged with warm (30°C) media containing drugs and the cells were further cultured at 30°C for 3 min. The cells were fixed with MeOH and stained by immunocytochemistry with anti- α -tubulin and anti-pericentrin for centrosomes. Gatastatin G2 clearly inhibited MT initiation from centrosomes dose-dependently in mitotic cells.



NOTE: All data in the manual were provided from Dr. Usui, University of Tsukuba.

Reference

1. Shintani *et al.*, *ACS Med. Chem. Lett.*, **11**, 1125-1129 (2020) Structure optimization of gatastatin for the development of γ -tubulin-specific inhibitor.
2. Chinen *et al.*, *Nat. Commun.*, **6**, 8722 (2015) The γ -tubulin-specific inhibitor gatastatin reveals temporal requirements of microtubule nucleation during the cell cycle.

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NucleoSeeing <Live Nucleus Green>

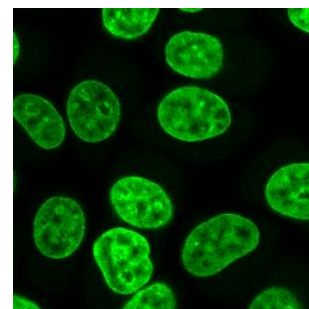
NucleoSeeing is DNA-responsive green dye for monitoring cell nucleus in live cells. As it shows low cytotoxicity and phototoxicity, it is very suitable for long-term live imaging of cell nucleus.

Catalog No. FDV-0029

Size 0.1 mg

Features

- Easy and quick procedure
- Compatible with 10% FBS
- Validated for both adherent cells and floating cells
- Little influence on cellular functions
- Ex/Em: 488 nm/520 nm (commercial FITC filters are available)



CytoSeeing <Reversible Cytoplasm Blue>

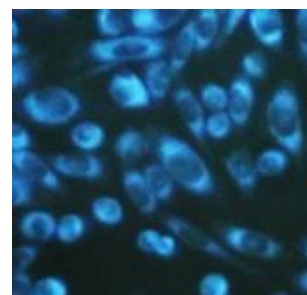
CytoSeeing is a reversible blue cytoplasm-staining dye for monitoring cell morphology. It allows observing cell structure and to reuse the cells after removing dye.

Catalog No. FDV-0017

Size 1 mg

Features

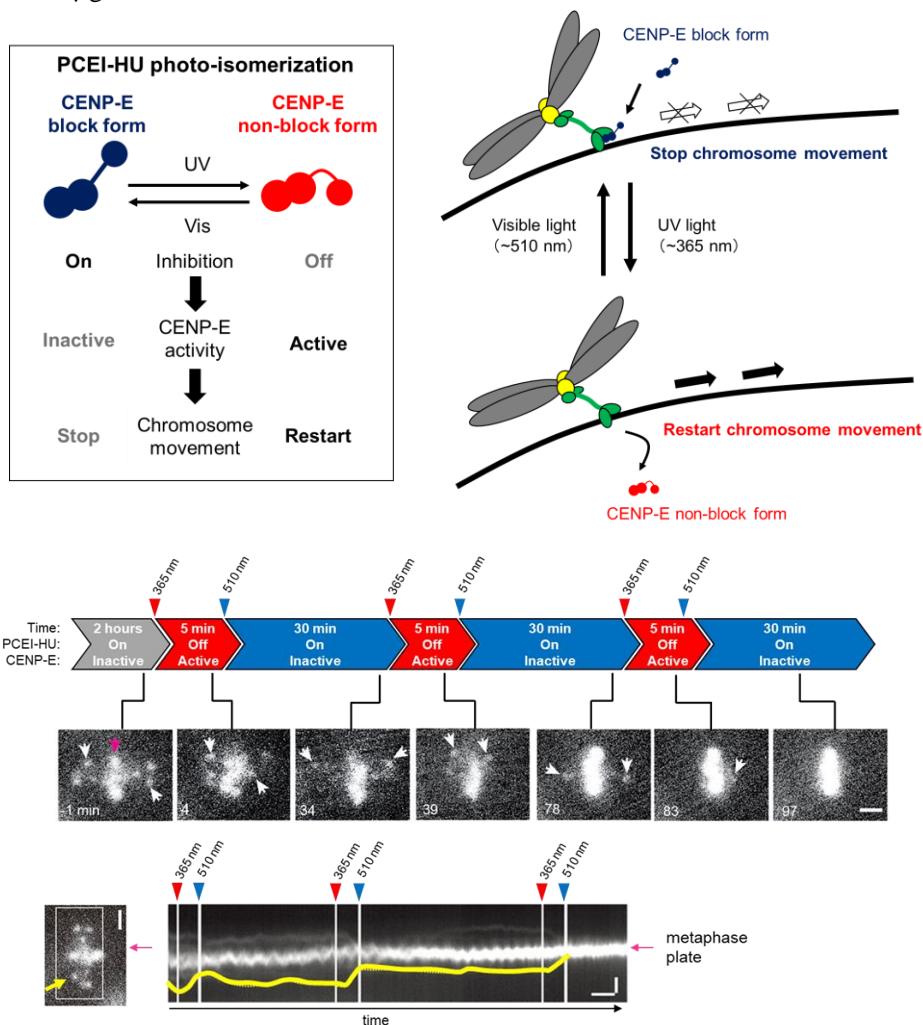
- Easy and quick staining less than 10 min
- Washable, reversible staining
- Validated for both adherent cells and suspension cells
- Little influence on cellular functions
- Ex/Em: 345 nm/456 nm



PCEI-HU <Photoswitchable CENP-E Inhibitor>

PCEI-HU is the world's first reversibly photoswitchable inhibitor for CENP-E, a representative motor protein of chromosome movement. This reagent repeatedly regulates chromosome movement along the spindle fiber by UV and visible light irradiation. PCEI-HU is a powerful tool to investigate spindle assembly checkpoint (SAC) mechanism.

Catalog No. FDV-0036
Size 50 µg



Live cell imaging of mitotic chromosomes in PCEI-HU treated LLC-PK1 cells

LLC-PK1 cells were treated with near-infrared DNA staining dye (SiR-DNA, 1 µM) and subsequently 1 µM PCEI-HU and 20 µM MG132 for 2 hours in darkroom. Cells were irradiated with UV light (365 nm) and Vis (510 nm) repeatedly and monitored in live-cell (Upper figure). Movement of a specific chromosome (yellow arrow) was analyzed in kymograph (Lower figure). While after UV irradiation, chromosomes moved to metaphase plate, but after Vis irradiation chromosomes left from metaphase plate. The chromosome was repeatedly regulated by UV/Vis cycles until chromosomes reaching to the metaphase plate.