# Data Sheet

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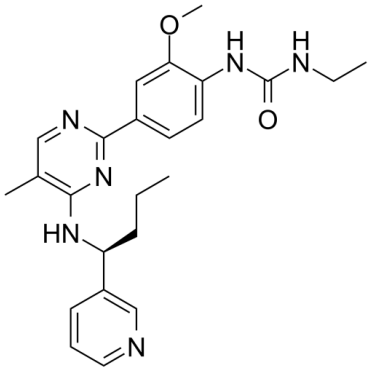
| **Product Name:** | Lexibulin |
| **Cat. No.:** | CS-3599 |
| **CAS No.:** | 917111-44-5 |
| **Molecular Formula:** | C24H30N6O2 |
| **Molecular Weight:** | 434.53 |
| **Target:** | Apoptosis; Microtubule/Tubulin |
| **Pathway:** | Apoptosis; Cell Cycle/DNA Damage; Cytoskeleton |
| **Solubility:** | DMSO : ≥ 100 mg/mL (230.13 mM); H2O : < 0.1 mg/mL (insoluble) |



## BIOLOGICAL ACTIVITY:

Lexibulin(CYT-997) is a potent tubulin polymerisation inhibitor with IC50 of 10-100 nM in cancer cell lines; with potent cytotoxic and vascular disrupting activity in vitro and in vivo. IC50 value: 10-100 nM(cell assay) [1] Target: tubulin polymerisation inhibitor in vitro: CYT997 prevented the in vitro polymerization of tubulin with an IC50 of ~3 μmol/L (compared with the half-maximal inhibitory concentration of 2 μmol/L for colchicine under identical conditions) as determined using the conventional turbidimetric assay for tubulin polymerization. CYT997 was also capable of reversibly disrupting the microtubule network in cells, visualized using fluorescence microscopy. Thus, treatment of A549 cells with CYT997 (1 μmol/L) lead to the rapid reorganization of microtubules, including the destruction of the existing microtubule network and accumulation of tubulin in plaques within the cytoplasm of some cells. After 24 hours, major alterations in cell morphology were evident, including loss of adhesion and cell rounding. The effect of 1 hour of treatment with CYT997 was reversible and cells rapidly recovered their normal microtubule architecture. Taken together, the data indicates that CYT997 belongs to the class of anticancer agents that disrupt, rather than stabilize, tubulin-containing structures. Although vehicle-treated cells show 15% and 19% in G2-M phase at 15 and 24 hours (respectively), cells treated with CYT997 (1 μ mol/L) had 38% and 43% of cells in G2-M at the same time points. Furthermore, at 24 hours post-CYT997 treatment, only 66% of total cells were in the G1, S, and G2-M phases, which suggests that cells blocked at the G2-M boundary do not exit back to G1, as in the normal cell cycle, but most likely are driven towards apoptosis and cell death [1]. Consistent with the disruption of cellular tubulin, CYT997 potently inhibits proliferation, induces cell cycle arrest and most importantly apoptosis of both human myeloma cell lines (HMCLs) and primary MM cells [2]. in vivo: In a xenograft model using the human prostate cancer cell line PC3, oral dosing of CYT997 was initiated 13 days after cell implantation by which time palpable tumors were evident. A dose-dependent inhibition of tumor growth was apparent with CYT997, which at the highest dose was equivalent to parenterally administered paclitaxel. A single dose of CYT997 (7.5 mg/kg i.p.) clearly decreased blood flow in liver metastases, and a significant reduction in blood flow was present 6 hours postdose [1]. CYT997 treatment (15 mg/kg/day) significantly prolongs the survival in a murine model of aggressive systemic myelomatosis [2].

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

Cell Proliferation Assay [1]: Cell proliferation was assessed (IC50, nmol/L) with either the Alamar blue or MTT assays after exposure to vehicle (0.1% DMSO) or CYT997 for 72 h. Cells were cultured in flat-bottomed 96-well plates at predetermined optimal densities and exposed to DMSO vehicle (0.1% final concentration) or varying concentrations of CYT997 for 72 h at 37°C. For MTT assays, 5 mg/mL of MTT was added to all wells, plates were incubated for 6 h at 37°C, and then lysis buffer was added (10% SDS in 0.01 N HCl) and absorbance was measured at 620 nm in a BMG Technologies Lumistar or Polarstar plate reader. For Alamar blue assays, Alamar blue (10 μL/well, AbD Serotec) was added to each well and the plates were incubated at 37°C for 4 h. The fluorescence was then measured using a fluorescence plate reader (BMG Technologies Polarstar) with an excitation filter at 544 nm and an emission filter at 590 nm. In vivo Tumor Models PC3 Xenograft Model [1]: Human PC-3 cells (106) were inoculated s.c. in the right ventral flank of male nude mice.

Subcutaneous tumor sizes were measured from day 8 postinoculation of cells, and mice were allocated at random to treatment groups on day 12. Administration of compounds (CYT997: 7.5, 15, or 30 mg/kg/d by oral gavage thrice a day; paclitaxel: 10 mg/kg i.v., thrice a week) or vehicle (NMP/PEG300/saline) commenced 13 d postinoculation of PC3 cells (treatment day 1). Dosing at 30 mg/kg/d was stopped after 11 treatment days due to excessive weight loss (>10%), and resumed after 7 d at 25 mg/kg/d for the remainder of the experiment (3 treatment days). The dimensions of subcutaneous PC3 tumors were measured thrice per week on alternate weekdays, and the experiment was ended on treatment day 21, when the tumors in control untreated mice reached 2 cm3.

## References:

[1]. Burns CJ, et al. CYT997: a novel orally active tubulin polymerization inhibitor with potent cytotoxic and vascular disrupting activity in vitro and in vivo. Mol Cancer Ther. 2009 Nov;8(11):3036-45.

[2]. Monaghan K, et al. CYT997 causes apoptosis in human multiple myeloma. Invest New Drugs. 2011 Apr;29(2):232-8.

## CAIndexNames:

Urea, N-ethyl-N'-[2-methoxy-4-[5-methyl-4-[[(1S)-1-(3-pyridinyl)butyl]amino]-2-pyrimidinyl]phenyl]-

## SMILES:

CCC[C@H](NC1=C(C)C=NC(C2=CC=C(NC(NCC)=O)C(OC)=C2)=N1)C3=CN=CC=C3



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