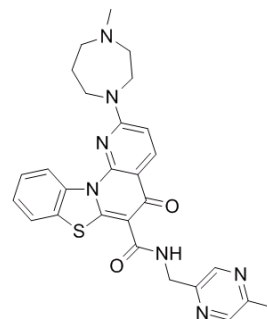


Data Sheet

Product Name:	CX-5461
Cat. No.:	HY-13323
CAS No.:	1138549-36-6
Molecular Formula:	C ₂₇ H ₂₇ N ₇ O ₂ S
Molecular Weight:	513.61
Target:	DNA/RNA Synthesis
Pathway:	Cell Cycle/DNA Damage
Solubility:	DMSO: < 5.3 mg/mL



BIOLOGICAL ACTIVITY:

CX-5461 is an inhibitor of **rRNA synthesis**, and selectively inhibits Pol I-driven transcription of rRNA with **IC₅₀** of 142 nM in HCT-116, A375, and MIA PaCa-2 cells, with no effect on Pol II, and possesses 250- to 300-fold selectivity for inhibition of rRNA transcription versus DNA replication and protein translation.

IC₅₀ & Target: IC₅₀: 142 nM (rRNA synthesis)

In Vitro: CX-5461 is found to selectively inhibit rRNA synthesis (Pol I IC₅₀=142 nM; Pol II IC₅₀ > 25 μM; selectivity appr 200-fold) in the HCT-116 cells. Selective inhibition of rRNA synthesis by CX-5461 is confirmed in two other human solid tumor cell lines; melanoma A375 (Pol I IC₅₀=113 nM; Pol II IC₅₀ > 25 μM) and pancreatic carcinoma MIA PaCa-2 (Pol I IC₅₀=54 nM; Pol II IC₅₀ appr 25 mM). CX-5461 possesses 250- to 300-fold selectivity for inhibition of rRNA transcription versus DNA replication and protein translation. CX-5461 exhibits broad antiproliferative potency in a panel of cancer cell lines in human cancer cell lines, but has minimal effect on viability of nontransformed human cells. The median EC₅₀ across all tested cell lines is 147 nM, yet all normal cell lines have EC₅₀ values of approximately 5, 000 nM. Evaluation of the antiproliferative dose response for HCT-116, A375, and MIA PaCa-2 cell lines yield EC₅₀ values of 167, 58, and 74 nM. CX-5461 induces autophagy and senescence in solid tumor cancer cells, rather than apoptosis, through a p53-independent process^[1]. Eμ-Myc lymphoma cells from tumor-bearing mice are exquisitely sensitive to CX-5461 with an IC₅₀ of 27.3 nM±8.1 nM for Pol I transcription after 1 hr and IC₅₀ of 5.4 nM ±2.1 nM for cell death after 16 hr. CX-5461 activates p53 via the nucleolar stress response in Eμ-MycLymphoma Cells^[2].

In Vivo: CX-5461 is orally bioavailable and demonstrates in vivo antitumor activity against human solid tumors in murine xenograft models. CX-5461 demonstrates significant MIA PaCa-2 TGI with TGI equal to 69% on day 31, comparable to that of gemcitabine (63% TGI). Gemcitabine is a positive control which is administered intraperitoneally once every 3 days at 120 mg/kg. Likewise, CX-5461 demonstrates significant A375 TGI with TGI equal to 79% on day 32^[1]. CX-5461 (50 mg/kg) inhibits the Eμ-Myc tumor cells with 84% repression in Pol I transcription at 1 hr posttreatment. CX-5461 induces a rapid reduction in tumor burden in the lymph nodes and a concomitant reduction of spleen size to within the normal range^[2].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: ^[1]Two short-lived RNA transcripts (half-lives appr 20-30 minutes), one produced by Pol I and another by Pol II, are quantitated by qRT-PCR as a measure of CX-5461-related effects on transcription. The 45S pre-rRNA serves as the Pol I transcript and the mRNA for the protooncogene c-myc serves as the comparator Pol II transcript. Both Pol I and Pol II transcription are known to be affected by general cellular stress. To minimize the potential effects of such stress, cells are exposed to test agents for only a short period of time (2 hours). This is sufficient time for these transcripts to be reduced by greater than 90% if CX-5461 affects their synthesis.

Cell Assay: ^[1]Cells are plated on 96-well plates and treated the next day with dose response of drugs for 96 hours. Cell viability is

determined using Alamar Blue and CyQUANT assays.

Animal Administration: [1] Animal experiments are performed with 5- to 6-week-old female athymic (NCR nu/nu fisol) mice of Balb/c. Mice are inoculated with 5×10^6 in 100 μL of cell suspension subcutaneously in the right flank. Tumor measurements are performed by caliper analysis, and tumor volume is calculated using the formula $(l \times w^2)/2$, where w =width and l =length in mm of the tumor. established tumors (appr 110–120 mm^3) are randomized into vehicle (50 mM NaH_2PO_4 , pH 4.5), gemcitabine, or CX-5461 treatment groups. Tumor growth inhibition (TGI) is determined on the last day of study according to the formula: $\text{TGI (\%)} = [100 - (V_f^D - V_i^D) / (V_f^V - V_i^V) \times 100]$, where V_i^V is the initial mean tumor volume in vehicle-treated group, V_f^V is the final mean tumor volume in vehicle-treated group, V_i^D is the initial mean tumor volume in drug-treated group, and V_f^D is the final mean tumor volume in drug-treated group.

References:

- [1]. Drygin D et al. Targeting RNA polymerase I with an oral small molecule CX-5461 inhibits ribosomal RNA synthesis and solid tumor growth. *Cancer Res.* 2011 Feb 15;71(4):1418–30.
- [2]. Bywater MJ, et al. Inhibition of RNA Polymerase I as a Therapeutic Strategy to Promote Cancer-Specific Activation of p53. *Cancer Cell.* 2012 Jul 10; 22(1):51–65.

Caution: Product has not been fully validated for medical applications. For research use only.

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