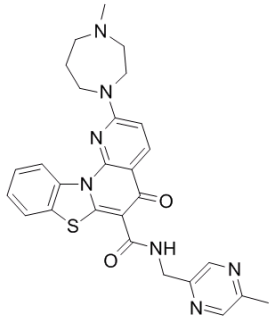
Data Sheet

Product Name:	CX–5461
Cat. No.:	HY-13323
CAS No.:	1138549-36-6
Molecular Formula:	$C_{27}H_{27}N_7O_2S$
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 IC_{50}=142 nM; Pol II IC_{50} > 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 IC_{50}=113 nM; Pol II IC_{50} > 25 μM) and pancreatic carcinoma MIA PaCa–2 (Pol I IC_{50}=54 nM; Pol II IC_{50} 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 EC_{50} across all tested cell lines is 147 nM, yet all normal cell lines have EC_{50} values of approximately 5, 000 nM. Evaluation of the antiproliferative dose response for HCT–116, A375, and MIA PaCa–2 cell lines yield EC_{50} 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 IC_{50} of 27.3 nM±8.1 nM for Pol I transcription after 1 hr and IC_{50} 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: TGI (%)=[100 - (Vf^D- Vi^D)/ (Vf^V - Vi^V) × 100], where Vi^V is the initial mean tumor volume in vehicle–treated group, Vf^V is the final mean tumor volume in vehicle–treated group, Vi^D is the initial mean tumor volume in drug–treated group, and Vf^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.

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