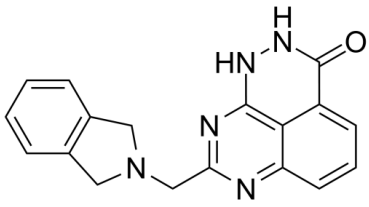
Data Sheet

Product Name:	E7449
Cat. No.:	CS-3479
CAS No.:	1140964-99-3
Molecular Formula:	$C_{18}H_{15}N_5O$
Molecular Weight:	317.34
Target:	PARP
Pathway:	Cell Cycle/DNA Damage; Epigenetics
Solubility:	DMSO : 6.4 mg/mL (20.17 mM; Need ultrasonic and warming)



BIOLOGICAL ACTIVITY:

E7449 is a potent **PARP1** and **PARP2** inhibitor and also inhibits **TNKS1** and **TNKS2**, with **IC_{50}**s of 2.0, 1.0, □50 and □50 nM for PARP1, PARP2, TNKS1 and TNKS2, respectively, using ^{32}P-NAD$^+$ as substrate. IC50 & Target: IC50: 2.0 nM (PARP1), 1.0 nM (PARP2), □50 nM (TNKS1), □50 nM (TNKS2)[1] **In Vitro:** E7449 is a potent PARP1 and PARP2 inhibitor and also inhibits TNKS1 and TNKS2, with IC_{50}s of 2.0, 1.0, □50 and □50 nM for PARP1, PARP2, TNKS1 and TNKS2, respectively, using ^{32}P-NAD$^+$ as substrate. E7449 shows no obvious inhibiotry effects on PARP3 or PARPs 6-16. E7449 traps PARP1 onto damaged DNA, and affects DNA repair pathways beyond homologous recombination (HR). E7449 most potnetly suppresses cells deficient in components of the HR pathway (BRCA1 and 2, CtIP, Rad54). E7449 (10 μM) inhibits Wnt signaling in SW480 cells[1]. **In Vivo:** E7449 moderately inhibits the growth of tumors at 100 mg/kg, and significantly ehhances the inhibition via 10, 30 and 100 mg/kg oral dosing in combination with temozolomide (TMZ) in the mouse melanoma B16-F10 isograft model. E7449 (30 or 100 mg/kg, p.o.) inhibits PARP, shows anti-tumor activity, and is well-tolerated without any obvious body weight loss or deaths in a BRCA mutant xenograft model. E7449 (30, 100 or 300 mg/kg, p.o.) suppresses re-growth of hair in a dose dependent manner, and blocks Wnt signaling in C57BL/6 mice. E7449 (100 mg/kg, p.o.) combined with MEK inhibitor exhibits antitumor activity in a Wnt1 subcutaneous model (mammary tumors initially isolated from Wnt1 (int-1) transgenic mice)[1].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [1]Briefly, 24 to 48 h after transfection, cells are washed 3× in ice-cold PBS and lysed for 20 min on ice in cell lysis buffer (CLB: 50 mM HEPES, pH 7.4, 150 mM NaCl, 1 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 1% TritonX-100, 1 μg/mL leupeptin, aprotinin, pepstatin, PMSF). Lysates are subject to ultracentrifugation at 100,000 g for 30 min. Cleared lysates are incubated for 1 h at 4°C with anti-GFP antibody (3E6) and pre-bound protein A magnetic beads. Beads are then washed 1 × 5 min in CLB, followed by 3 × 10 min washes in CLB containing 1 M NaCl, and 1 × 5 min wash in PARP reaction buffer (PRB; 50 mM Tris, pH 7.5, 50 mM NaCl, 0.5 mM DTT, 0.1% TritonX-100, 1 μg/mL leupeptin, aprotinin, pepstatin). **NAD$^+$ incorporation reactions** are performed in PRB containing 10 μM NAD$^+$ supplemented with ^{32}P-NAD$^+$ at 1:20 ratio for 30 min at 25°C. For **PARPs** with low incorporation signals (PARP4, 5a and 16), NAD$^+$ incorporation is performed at 1:5 ratio for 1 h at 25°C. Beads are then re-suspended in Laemmli sample buffer, heated to 65°C for 10 min, the beads removed using a magnet, and the supernatant spotted onto Whatman paper. Samples are analyzed via phosphorimaging[1]. **Cell Assay:** [1]Proliferation assays are performed in a panel of **32 isogenic DT40 cell lines**, in which each line is deficient in a distinct DNA repair gene. Cells are seeded and incubated with test compound at various concentrations for 2-3 days (□ 8 cell cycles). Cell growth is assessed using **XTT** and IC_{50} values are calculated using the GraphPad Prism 5 software version 5.02. Each experiment is conducted in duplicate and a minimum of 3 separate experiments are performed. Human breast cancer cell lines, HCC1143, HCC70, HCC1806, MDA-MB-436, T47D, MDA-MB-157, MDA-MB-231, MDA-MB-468,

MDA-MB-453, BT-20 and Hs578T are used. For cell line panel assays, cells are maintained and assayed in RPMI 1640 or DMEM medium containing 10% FBS. For proliferation assays cells are plated at low density in 96 well plates. **E7449 is added at various concentrations** and plates incubated for a total of 8 days; compound and medium are replenished on day 4. Cell growth is assessed using the CellTiter-Glo cell viability assay. Each experiment is conducted in duplicate and a minimum of 3 separate experiments are performed[1]. **Animal Administration:** E7449 is formulated in 0.5% methyl cellulose in water.[1]Temozolomide (TMZ) combination in B16-F10 isograft model: **female C57BL/6 mice** are inoculated subcutaneously with **B16-F10 cells (2 × 10^5)**. Following randomization by body weight, drug treatment is initiated 1 day post-inoculation. Both **E7449** and TMZ are **formulated in 0.5% methyl cellulose and orally administrated once per day**. TMZ is administered daily on days 1 to 5 at 50 mg/kg as a single agent or in combination. **E7449 is administered daily on days 1 to 7 at doses of 10, 30 and 100 mg/kg** in combination with TMZ and at a dose of 100 mg/kg as a single agent. The control group is treated with vehicle (0.5% methyl cellulose in water). E7449 or vehicle is administered first and when dosing of all animals is complete TMZ is administered to animals receiving the combination[1].

References:

[1]. McGonigle S, et al. E7449: A dual inhibitor of PARP1/2 and tankyrase1/2 inhibits growth of DNA repair deficient tumors and antagonizes Wnt signaling. Oncotarget. 2015 Dec 1;6(38):41307-23.

CAIndexNames:

3H-Pyridazino[3,4,5-de]quinazolin-3-one, 8-[(1,3-dihydro-2H-isoindol-2-yl)methyl]-1,2-dihydro-

SMILES:

O=C1NNC2=C3C1=CC=CC3=NC(CN4CC5=C(C=CC=C5)C4)=N2

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