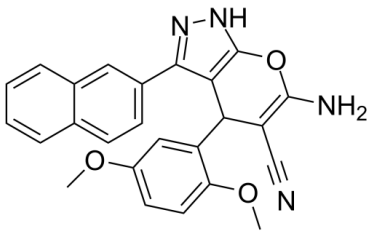
Data Sheet

Product Name:	RBC8
Cat. No.:	CS-4199
CAS No.:	361185-42-4
Molecular Formula:	C25H20N4O3
Molecular Weight:	424.45
Target:	Ras
Pathway:	GPCR/G Protein
Solubility:	H2O : < 0.1 mg/mL (insoluble); DMSO : ≥ 40 mg/mL (94.24 mM)



BIOLOGICAL ACTIVITY:

RBC8 is a novel small molecule inhibitor of Ral GTPase; has IC50 of 3.5 μM in H2122 cell and 3.4 μM in H358 cell. IC50 value: Target: Ral GTPase inhibitor RBC8 or BQU57 treatment showed no further inhibition of colony formation after Ral knockdown. RBC8 and BQU57 showed favorable properties that define good drug candidates. To test the effect of Ral inhibitors on xenograft tumor growth, nude mice were inoculated subcutaneously with H2122 human lung cancer cells and treated intraperitoneally with 50 mg/kg/d of RBC8 for 21 days (except weekends). RBC8 inhibited tumor growth to a similar extent as dual knockdown of RalA and RalB.

PROTOCOL (Extracted from published papers and Only for reference)

Cell assay [1] Cells were seeded into 6-well plates (coated with a base layer made of 2.0 ml of 1% low-melting-point agarose) at 15,000 cells per well in 3.0 ml of 0.4% low-melting-point agarose containing various concentration of drug. Two to four weeks (depending on cell line) after incubation, cells were stained with 1.0 mg/ml Nitro Blue Tetrazolium and colonies were counted under a microscope. The IC50 values were defined as the concentration of drug that resulted in 50% reduction in colony number compared to DMSO treated control. For growth effects induced by siRNA treatment, cells were transfected with 50 nM siRNA against RalA, RalB or both (RalA/B) using methods and sequences described8. After 48 hr, cells were subjected to the soft agar colony formation assay as describe above. For the chemo-genetic experiments, siRNA treated cells were seeded into soft agar in the presence of various concentrations of drug. For the overexpression experiments, H358 cells stably overexpressing FLAG, FLAG-RalAG23V or FLAG-RalBG23V were generated and cells were subjected to the soft agar colony formation assay in the presence of drug. Attempts to stably overexpress FLAG-RalAG23V or FLAG-RalBG23V in H2122 cells were unsuccessful and the rescue experiments with H2122 were carried out 48 hr after transient transfection with FLAG, FLAG-RalAG23V or FLAG-RalBG23V using agar colony formation assay in the presence of drug. Animal administration [1] Nude mice were inoculated with 5 × 106 cells H2122 cells s.c. When tumor reached an average of 250 mm3, mice were randomized into 6 per group (no blinding was done) and were given an i.p. dose of RBC8 or BQU57 at various concentrations. Tumors were then collected 3h after injection of RBC8 or BQU57.

References:

[1]. Yan C, et al. Discovery and characterization of small molecules that target the GTPase Ral. Nature. 2014 Nov 20;515(7527):443-7.

CAIndexNames:

Pyrano[2,3-c]pyrazole-5-carbonitrile, 6-amino-4-(2,5-dimethoxyphenyl)-1,4-dihydro-3-(2-naphthalenyl)-

SMILES:

N#CC(C1C2=CC(OC)=CC=C2OC)=C(N)OC3=C1C(C4=CC=C5C=CC=CC5=C4)=NN3

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