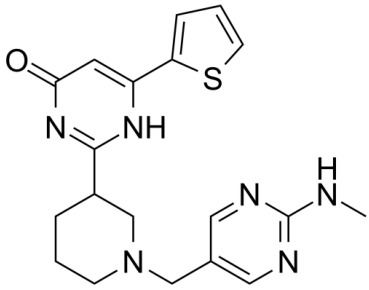
Data Sheet

Product Name:	Ribocil
Cat. No.:	CS-5272
CAS No.:	1381289-58-2
Molecular Formula:	C19H22N6OS
Molecular Weight:	382.48
Target:	Bacterial
Pathway:	Anti-infection
Solubility:	DMSO : ≥ 24.8 mg/mL (64.84 mM)



BIOLOGICAL ACTIVITY:

Ribocil is a highly selective chemical modulator of bacterial riboflavin riboswitches. Ribocil strongly inhibits GFP expression, achieving a 50% effective concentration (EC50) of 0.3 μM. Target: in vitro: Ribocil is a highly specific bioactive synthetic mimic of FMN, which competes with the natural ligand to inhibit FMN riboswitch-mediated expression of ribB and inhibits bacterial growth. Ribocil-B demonstrates superior microbiological activity as compared to Ribocil-A (minimum inhibitory concentration (MIC) = 1 μg/ml versus MIC ≥ 64 μg/ml), inhibition of riboflavin synthesis (IC50 = 0.13 μM versus IC50 > 26 μM), and binding affinity to the E. coli FMN aptamer (Kd = 6.6 nM versus Kd ≥ 10,000 nM).[1]

PROTOCOL (Extracted from published papers and Only for reference)

Cell assay [1] Overnight cultures of MB5746 or MB5746 RibocilR cells were diluted 1:50 in CAMHB and distributed (1.25 ml) into 10-ml culture tubes containing diluted Ribocil (twofold dilution series) or DMSO (1%) as mock control. The treated cultures were incubated with shaking at 37°C for about 20 h, after which the OD600 of the culture was determined and 500 μl was moved to a 96-well deep-well plate. After centrifugation (4,000 r.p.m.) for 10 min, the bacterial cell pellets were rinsed with lysozyme dilution buffer (10 mM Tris HCl (pH 8.0), 25 mM NaCl, 1 mM EDTA) and centrifuged again. Cell pellets were then re-suspended in 100 μl of lysozyme solution (10 mg/ml lysozyme in lysozyme dilution buffer), incubated at 37°C for 30 min, and then frozen at -20 °C. Riboflavin, FMN and FAD concentrations in the bacterial lysates were determined using the Vitamin B2 HPLC detection kit and Vitamin B2 column following the procedure recommended by the manufacturer scaled for a 50 μl sample (bacterial lysate). A Shimadzu HPLC system with fluorescence detector was used at a flow rate of 1.0 ml/min and flavin detection was carried out at 450 nm. Flavin levels were determined for an equivalent number of cells by correcting raw AUCs using the OD600 ratio of the treated versus the untreated cultures. Animal administration [1] Twelve-week-old female DBA2/J mice were chosen for this study based on weight (~20 g) and combined into one pool. Animals were then randomly selected from this pool and placed in groups of five in separate boxes. Subjects were treated by intraperitoneal (i.p.) injection with 150 mg/kg of cyclophosphamide on day -4 and 100 mg/kg on day -1. On day 0, mice were inoculated i.p. with 0.5 ml of bacteria in 3% mucin (5.0 × 104 CFU ml-1) or a higher inoculum of 5.0 × 105 CFU ml-1. Thirty minutes post-inoculation, mice (n = 5 per group) were treated by subcutaneous (s.c.) injection three times over 24 h with either ciprofloxacin (0.5 mg/kg, Ribocil-C (at either 120, 60, or 30 mg/kg)) or 10% DMSO sham. On day 1, subjects were euthanized via CO2 asphyxiation and spleens were aseptically removed, weighed and homogenized in 1.5 ml of sterile saline with 10% glycerol. Tissue homogenates were serially diluted tenfold in sterile saline and selected concentrations were plated on TSA II (5% sheep's blood) agar plates. Plates were incubated at 35°C for 24 h and CFU per g of spleen tissue were determined.

References:

[1]. Howe JA, et al. Selective small-molecule inhibition of an RNA structural element. Nature. 2015 Oct 29;526(7575):672-7.

CAIndexNames:

4(3H)-Pyrimidinone, 2-[1-[[2-(methylamino)-5-pyrimidinyl]methyl]-3-piperidinyl]-6-(2-thienyl)-

SMILES:

C1(C=C(NC(=N1)C1CN(CC2C=NC(NC)=NC=2)CCC1)C1=CC=CS1)=O

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