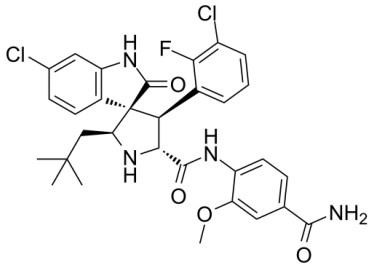
Data Sheet

Product Name:	RO8994
Cat. No.:	CS-5002
CAS No.:	1309684-94-3
Molecular Formula:	C31H31Cl2FN4O4
Molecular Weight:	613.51
Target:	E1/E2/E3 Enzyme; MDM-2/p53
Pathway:	Apoptosis; Metabolic Enzyme/Protease
Solubility:	DMSO : ≥ 45 mg/mL (73.35 mM)



BIOLOGICAL ACTIVITY:

RO8994 is a highly potent and selective series of spiroindolinone small-molecule MDM2 inhibitor, with IC50 of 5 nM (HTRF binding assays) and 20 nM (MTT proliferation assays). IC50 value: 5 nM (in HTRF binding assays), 20 nM (in MTT proliferation assays) Target: MDM2 in vitro: RO8994 represents a new generation of p53-MDM2 antagonists with marked improvement in pharmacological properties for potential clinical development. RO8994 induces dose-dependent up-regulation of p53 target genes and apoptosis in wild-type p53 cancer cells, consistent with its non-genotoxic mechanism of p53 activation. in vivo: RO8994 displays remarkable tumor growth inhibition in the wild-type p53, MDM2-amplified SJSA-1 osteosarcoma tumor xenograft model - exhibiting significant (>60%) tumor growth inhibition at the low dose of 1.56 mg/kg, tumor stasis at 3.125 mg/kg and regression at 6.25 mg/kg.

PROTOCOL (Extracted from published papers and Only for reference)

Kinase assay [1] The p53-MDM2 HTRF assay was performed in buffer containing 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM DTT, 0.02 or 0.2 mg/ml BSA. Small-molecule inhibitors were stored in aliquots as10 mM stock solution in DMSO at 4℃ in 96-deep-well plate. It was thawed and mixed immediately prior to testing. The compound was incubated with GST-MDM2 and a biotinylated p53 peptide for one hour at 37℃. Phycolink goat anti-GST (Type 1) allophycocyanin and Eu-8044-streptavidin were then added and followed by one hour incubation at room temperature. Plates were read using the Envision fluorescence reader. IC50 values were determined from inter-plate duplicate or triplicate sets of data. Cell assay [1] Each cell line in its optimal medium was plated at the appropriate seeding density to give logarithmic growth over the course of the assay in a 96-well tissue culture plate. Plates were incubated overnight at 37℃ in a humidified incubator with 5% CO2. The next day, RO8994 was serially diluted 1:3 in the appropriate medium containing 3% DMSO. One-tenth final volume of each dilution was added in duplicate to the plates containing cells. The same volume of 3% DMSO in medium was added to a row of control wells. Thus, the final concentration of DMSO in all wells was 0.3%. The plates were returned to the incubator, and at set time points plates were analysed as follows: MTT was added to each well to yield a final concentration of 1 mg/ml. Plates were returned to the incubator for 2.5 hours. The MTT-containing medium was removed and the resulting formazan metabolite was solubilized in 100% ethanol with shaking for 15 minutes at room temperature. Absorbances were read in a microtiter plate reader at a wavelength of 570 nm with a 650 nm reference.

References:

[1]. Zhang Z, et al. Discovery of potent and selective spiroindolinone MDM2 inhibitor, RO8994, for cancer therapy. Bioorg Med Chem. 2014 Aug 1;22(15):4001-4009.

CAIndexNames:

Spiro[3H-indole-3,3'-pyrrolidine]-5'-carboxamide, N-[4-(aminocarbonyl)-2-methoxyphenyl]-6-chloro-4'-(3-chloro-2-fluorophenyl)-2'-(2,2-dimethylpropyl)-1,2-dihydro-2-oxo-, (2'S,3R,4'S,5'R)-

SMILES:

O=C([C@H](N[C@H]1CC(C)(C)C)[C@H](C2=CC=CC(Cl)=C2F)[C@@]31C(NC4=C3C=CC(Cl)=C4)=O)NC5=CC=C(C(N)=O)C=C5OC

Tel: 732-484-9848 Fax: 888-484-5008 E-mail: sales@ChemScene.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA