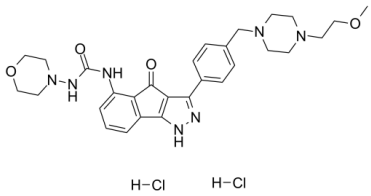
Data Sheet

Product Name:	RGB-286638
Cat. No.:	CS-3453
CAS No.:	784210-87-3
Molecular Formula:	$C_{29}H_{37}Cl_2N_7O_4$
Molecular Weight:	618.55
Target:	CDK; GSK-3; JAK; MEK
Pathway:	Cell Cycle/DNA Damage; Epigenetics; JAK/STAT Signaling; MAPK/ERK Pathway; PI3K/Akt/mTOR; Stem Cell/Wnt
Solubility:	DMSO : ≥ 150 mg/mL (242.50 mM)



BIOLOGICAL ACTIVITY:

RGB-286638 is a **CDK** inhibitor that inhibits the kinase activity of **cyclin T1-CDK9**, **cyclin B1-CDK1**, **cyclin E-CDK2**, **cyclin D1-CDK4**, **cyclin E-CDK3**, and **p35-CDK5** with **IC_{50}**s of 1, 2, 3, 4, 5 and 5 nM, respectively; also inhibits GSK-3β, TAK1, Jak2 and MEK1, with **IC_{50}**s of 3, 5, 50, and 54 nM. IC50 & Target: IC50: 1 nM (CDK9), 2 nM (CDK1), 3 nM (CDK2), 4 nM (CDK4), 5 nM (CDK3), 5 nM (CDK5), 44 nM (cyclin H-CDK7), 55 nM (CDK6/cyclin D3), 3 nM (GSK-3β), 5 nM (TAK1), 50 nM (Jak2), 54 nM (MEK1) [1] **In Vitro:** RGB-286638 is an indenopyrazole-derived CDK inhibitor (CDKI) with K_i-nanomolar activity against transcriptional CDKs. RGB-286638 inhibits several tyrosine and serine/threonine non-CDK enzymes, i.e. GSK-3β, TAK1, AMPK, Jak2, MEK1. The dose- and time-dependent effect of treatment with RGB-286638 (12.5-100nM) is investigated on the growth of human p53-wt (MM.1S, MM.1R, and H929) and p53-mutant (U266, OPM1, and RPMI) MM cells by MTT assay, assessing viability at 24 and 48 hours. The half-maximally effective concentrations (EC_{50}) range between 20 and 70 nM at 48 hours. Dose-dependent differences in growth among p53-wt and -mutant cells are observed after 50nM treatment, with p53-wt MM.1S, MM.1R and H929 being slightly more sensitive to RGB-286638 treatment at 48h[1]. **In Vivo:** Dose-finding studies with RGB-286638 identify 40 mg/kg/day IV treatment as the maximum tolerated dose in SCID mice. Five days IV treatment with RGB-286638 significantly suppresses MM tumor growth, with maximum TGI (%) noted at day 14 following end of treatment at 85.06% and 86.34% in the 30 mg/kg and 40 mg/kg treated cohorts respectively. The log10 cell kill (LCK Td: 4.5 days) is 1.6 for both treated groups. RGB-286638 treatment is also associated with improved survival, evidenced by first death at day 24 in controls versus day 43 in both treated groups. No toxic deaths occurred during this study: maximum percentage of body weight (BW) loss is observed on day 5 (8.4%) at 30 mg/kg dosage schedule, and on day 15 (9.9%) after 40 mg/kg dosing, with weight recovery in the following two weeks[1].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [1]Nuclear proteins are isolated from MM.1S cells treated with 50nM RGB-286638 for 1, 4, and 8 h, using Nuclear Extraction Kit. Nuclear protein aliquots are added to the 96-well plate coated with specific double-stranded DNA sequence containing the p53 response element for overnight incubation. p53 in the nuclear extract is detected by addition of a specific primary antibody directed against p53. A secondary antibody conjugated to HRP is added to provide a sensitive colorimetric readout at 450 nm. All experiments are performed in triplicates[1].

Cell Assay: RGB-286638 is provided in 20 mg/mL aqueous solution[1].[1]Colorimetric assays are performed to assay drug activity at increasing concentrations of RGB-286638 (0-100nM). Expressing wild-type p53 (MM.1S, MM.1R, H929) or mutant-p53 (U266, OPM1, RPMI) cells from 24- or 48-h cultures are pulsed with 10μL of 5mg/mL MTT to each well, followed by incubation at 37°C for 4h, and addition of 100 μL isopropanol containing 0.04 HCl. Absorbance readings at a wavelength of 570nm (with correction using readings at 630nm) are taken on a spectrophotometer. All experiments are performed in triplicates[1].

Animal Administration: RGB-286638 is prepared in 5% dextrose/water (D5W) pH5.2 (Mice)[1].[1]Mice[1]
The in vivo anti-MM activity of RGB-286638 is evaluated in an MM xenograft model. RGB-286638 dosing solutions of 2 and 3 mg/mL in 5% dextrose/water (D5W) pH5.2, as well as D5W pH5.2 for vehicle control dosing group, are prepared and provided by Agennix AG. CB-17 severe combined immunodeficient (SCID) mice are used. Forty male 5-6 week old mice are irradiated (2 Gy [200 rad]) using cesium 137 (137Cs)-irradiator source); 24h after irradiation, 2.5×10^6 MM.1S cells are inoculated subcutaneously in the upper back. When tumor weight is approximately 100 mg, mice are randomly assigned into 3 cohorts receiving daily IV tail vein injections for 5 consecutive days with either RGB-286638 30 mg/kg (8 mice), 40 mg/kg (9 mice), or control vehicle alone (10 mice). Animals are monitored for body weight and tumor volume by caliper measurements every alternate day. Tumor volume is estimated. Survival is evaluated from the first day of treatment until death. Tumor growth is evaluated using caliper measurements from the first day of treatment until day of first sacrifice. Percentage tumor growth inhibition (TGI) is calculated.

References:

[1]. Cirstea D, et al. Small-molecule multi-targeted kinase inhibitor RGB-286638 triggers P53-dependent and -independent anti-multiple myeloma activity through inhibition of transcriptional CDKs. Leukemia. 2013 Dec;27(12):2366-75.

CAIndexNames:

Urea, N-[1,4-dihydro-3-[4-[[4-(2-methoxyethyl)-1-piperazinyl]methyl]phenyl]-4-oxoindeno[1,2-c]pyrazol-5-yl]-N'-4-morpholinyl-, hydrochloride (1:2)

SMILES:

O=C(NN1CCOCC1)NC2=CC=CC(C3=C4C(C5=CC=C(CN6CCN(CCOC)CC6)C=C5)=NN3)=C2C4=O.[H]Cl.[H]Cl

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