Characterization of a novel small molecule with potent anticancer activity

Gina Chun[1], Young Bok Lee[1], Chang Ho Ahn[1], Won-Jea Cho[2], Sang Kook Lee[3], Hwa-Jin Chung[3], Deog Joong Kim[1]

[1]Rexahn Pharmaceuticals, Inc. Rockville, MD, USA.

[2]College of Pharmacy, Chonnam National University, Gwangju, Korea. [3]College of Pharmacy, Seoul National University, Seoul, Korea

Abstract

A series of isoquinolineamine derivatives were synthesized and screened as potential anticancer agents. Among them, we identified one isoquinolineamine analogue with potential anticancer activity. This compound, RX-8243, inhibited the proliferation of a variety of cancer cells derived from many human solid tumors with IC_{50} values ranging from 14 nM to 71 nM. The compound also was more effective against paclitaxel, gemcitabine and cisplatin-resistant cancer cell lines when compared to the original cytotoxic cancer drugs. Treatment with this compound significantly inhibited the growth of tumors and enhanced tumor regression in a paclitaxel-resistant xenograft model. RX-8243 is well tolerated and had no effects on body weight compared to control animals. Mechanistic studies showed that cells treated with this compound resulted in the elevation of cytochrome c in the cytosol, the decrease of Bcl-2 and the activation of Caspase 9. Further studies showed inhibition of the expression of several signaling molecules important for cell proliferation (p-Erk, p-p38 and p-Akt) and also decreased protein levels of p-GSK3α/β and β-catenin. Therefore, our results suggest that RX-8243 could be a promising antitumor agent.

Materials & Methods

IC_{50} values: Cancer cells were plated in 96-well plates. After 24 hours, the cells were treated with various concentrations of drug in triplicate for 96 hours. After incubation, cells were fixed with 10% TCA solution. Cell density was determined by sulforhodamine B (SRB) assay to measure total cellular protein and the inhibitory 50% concentration (IC_{50}) was determined. Resistance index (RI) was obtained by dividing the IC_{50} value of the resistant cell line by the IC_{50} value of the nonresistant cell line and represents the drug's efficacy against drug resistant cell lines *vs.* the corresponding cancer cell lines (parental, nonresistant). The lower RI value implies better efficacy.

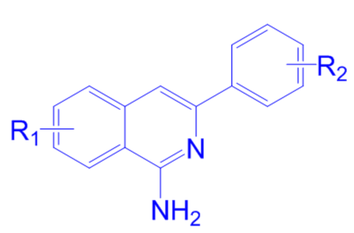
Xenograft model: Nude mice xenografted with human colon cancer HCT-15 cells (paclitaxel-resistant cell line) were treated with RX-8243. RX-8243 (10 mg/kg) and paclitaxel (10 mg/kg) was given by intraperitoneal injections three times per week starting at day 10 after implanting the tumor cells. Total body weight and tumor volume was measured at the indicated time points.

In vitro kinase activity: RX-8243 was tested against 64 kinases implicated in cancer progression through the Invitrogen SelectScreen™ services. Of those tested, RX-8243 inhibited Aurora A kinase with IC_{50} value of 0.37 μM.

Protein Expression: Protein lysates were extracted from HCT-15 cells treated with RX-8243 for 1 h, 24 h and 48 h and immunoblotting was performed. Immunoblots were probed with the following antibodies from Santa Cruz Biotechnology: cyctochrome c, Bcl-2, caspase 9, p-ERK, p-p38, p-Akt, Akt, p-β-catenin, β-catenin, p-GSK3α/β, GSK3α/β and β-actin for loading control.

Results

Chemical scaffold of RX-8243



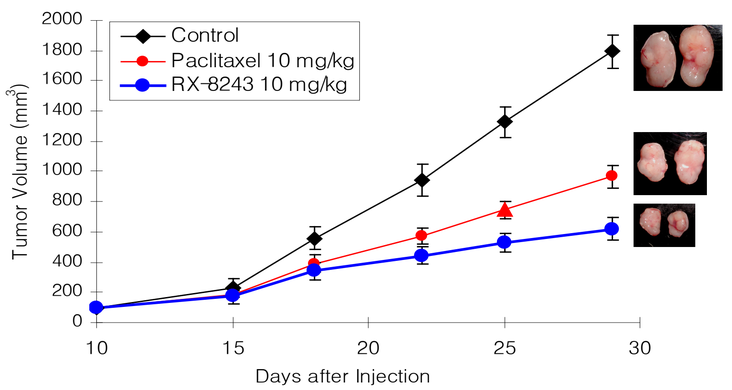
RX-8243 inhibits the proliferation of human cancer cell lines

Cell Line	Tissue	IC_{50} (μM)
MKN-45	Stomach	0.022
Caki-1	Kidney	0.022
OVCAR3	Ovary	0.014
U251	Brain	0.050
MDA-MB-231	Breast	0.021
HCT116	Colon	0.019
PC-3	Prostate	0.020
HeLa	Cervix	0.025
PANC-1	Pancreas	0.019
HepG2	Liver	0.071
A549	Lung	0.052
SK-MEL-28	Melanoma	0.032

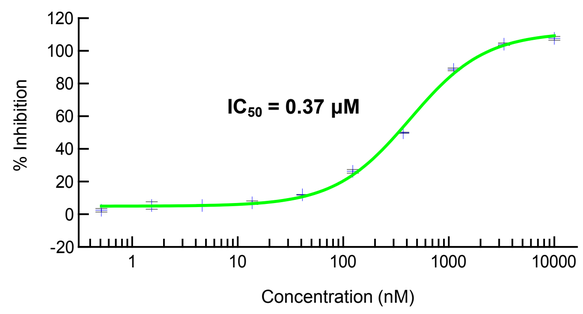
RX-8243 inhibits the proliferation of drug-resistant cancer cells

Compound	IC_{50} (μM)		Resistant Index (RI)
	HCT-116	**HCT-15-Tax**	
RX-8243	0.019	0.015	**0.79**
Paclitaxel	0.002	0.140	**70**
	A2780	**ADDP-Cis**	
RX-8243	0.012	0.014	**1.17**
Cisplatin	0.13	10.51	**80.85**
	A2780	**AG6000-Gem**	
RX-8243	0.012	0.010	**0.83**
Gemcitabine	0.012	13.80	**1150**

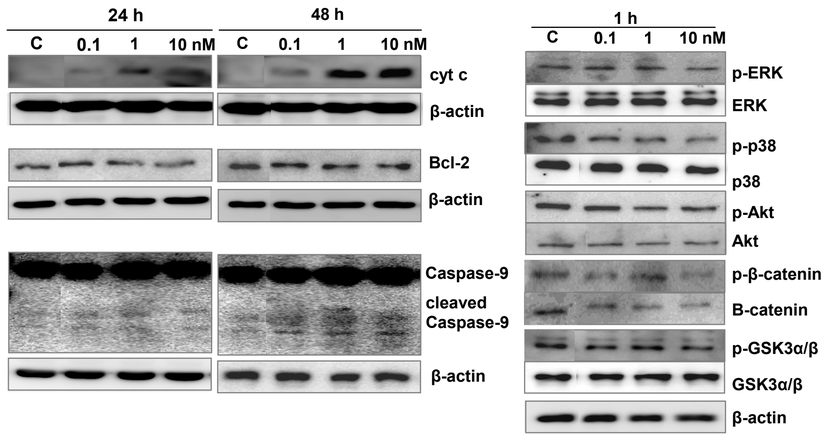
Reduction of tumor volume in paclitaxel-resistant xenograft model



RX-8243 inhibits Aurora A kinase



Alterations in the expression of proteins involved in cell death and survival



Summary

-Our compound, RX-8243 inhibited the proliferation of a variety of human cancer cells with IC_{50} values ranging from 14 nM to 71 nM

-RX-8243 inhibited cell proliferation of gemcitabine, cisplatin and taxol-resistant cells

-RX-8243 significantly decreased tumor growth in a paclitaxel-resistant xenograft model and was well tolerated (no affect on body weight compared to control animals, data not shown)

-RX-8243 inhibited the in vitro kinase activity of Aurora A by 97% at 1 μM, and with IC_{50} value of 0.37 μM

-Cells treated with RX-8243 resulted in the elevation of cytochrome c in the cytosol, decrease of Bcl-2 and the activation of Caspase 9.

-RX-8243 decreased the expression of several signaling molecules important for cell proliferation (p-Erk, p-p38 and p-GSK3α/β) and also decreased protein levels of p-Akt and β-catenin

Discussion

RX-8243, an isoquinolineamine analogue, is a novel small molecule in the treatment of cancer. We have found that this compound shows superior efficacy in a variety of human cancer cell lines as well as drug resistant cancer cells. Moreover, this compound inhibits the growth of tumor and enhances tumor regression in a paclitaxel-resistant xenograft model. Our results suggest that RX-8243 could be a promising antitumor agent.

For further information about RX-8243 and Rexahn Pharmaceuticals, Inc., please contact Dr. DJ Kim: kimdj@rexahn.com, (240) 268-5300 x 306