Anti-tumor activity of a new quinoxalinyl-piperazine compound (RX-5902)

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Abstract

Novel 1-[(5 or 6-substituted alkoxyquinoxalinyl)aminocarbonyl]-4-(hetero)arylpiperazine derivatives were synthesized and evaluated as anti-tumor agents. Despite their structural similarity to the quinoxalinyl-piperazine core scaffold, the IC50 values of the compounds against human cancer cells depended on the substitution at the quinoxaline ring as well as at the phenyl ring. The best compound, showing IC50 values ranging from 11 to 21 nM, was selected and characterized further both *in vitro* and *in vivo*. This compound was more potent against paclitaxel resistant HCT-15 colorectal cancer cells compared to paclitaxel itself. Combined treatment of this compound with known anti-cancer drugs, such as paclitaxel, doxorubicin, cisplatin, gemcitabine or 5-fluorouracil, displayed synergistic growth inhibition of cancer cells. In mice bearing tumor xenografts, treatment with the compound completely inhibited the growth of various human tumors, enhanced tumor regression without effects on body weight compared to control animals. Mechanistic studies have shown that this quinoxalinyl-piperazine compound is a G2/M-specific cell cycle inhibitor and inhibits anti-apoptotic Bcl-2 protein with p21 induction. The results clearly demonstrate that our new quinoxalinyl-piperazine compound could become a novel class of anti-tumor chemotherapeutics.

Materials & Methods

In vitro Cell studies: Cancer cells were plated in 96-well plates. After 24 hours, the cells were treated with various concentrations of compounds for 96 hours. Cell growth inhibition was measured by sulforhodamine B (SRB) assay and IC50s were obtained. Resistance index (RI) was determined by dividing the IC50 value of the resistant cell line by the IC50 value of the nonresistant cell line.

Xenograft model: Xenograft studies in nude mice were performed with implanted human cancer cells. RX-5902 was administered orally with 5 days on/2 days off schedule, for 3 weeks. Total body weight and tumor volume were measured at the indicated time points.

Survival: Kaplan-Meier plots were performed

Cell cycle and Apoptosis: Cell cycle analysis and apoptosis studies were performed using MDA-MB-231 cells.

PK studies: Pharmacokinetics of RX-5902 was studied using Sprague-Dawley rats and Beagle Dogs.

Chemical scaffold of RX-5902

Results

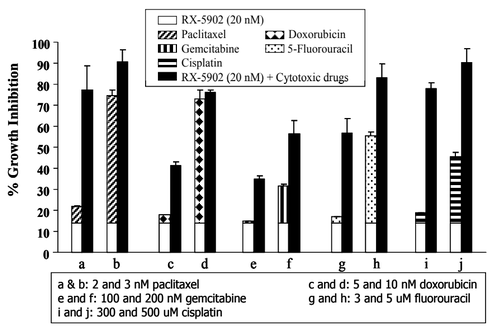
Inhibition of human cancer cell lines by RX-5902

Cell Line	Tissue	IC_{50} (nM)
Caki-1	Kidney	11
MDA-MB-231	Breast	12
OVCAR-3	Ovary	12
HepG2	Liver	19
HCT-116	Colon	19
SNB-19	Brain	20
SK-MEL-28	Melanoma	20
MKN-45	Stomach	20
HeLa	Cervix	21
A549	Lung	21
PC-3	Prostate	21
PANC-1	Pancreas	21

Inhibition of drug-resistant cancer cells by RX-5902

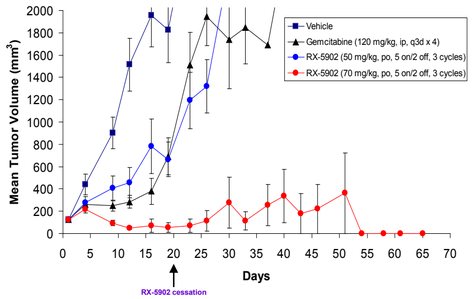
Compound	IC50 (nM)		Resistant Index (RI)
	HCT-116	**HCT-15-Tax**	
RX-5902	29	21	0.72
Paclitaxel	2	140	70
	A2780	**ADDP-Cis**	
RX-5902	48	17	0.35
Cisplatin	130	610	4.69
	A2780	**AG6000-Gem**	
RX-5902	48	26	0.54
Gemcitabine	4	25000	6250

Synergistic effects of RX-5902

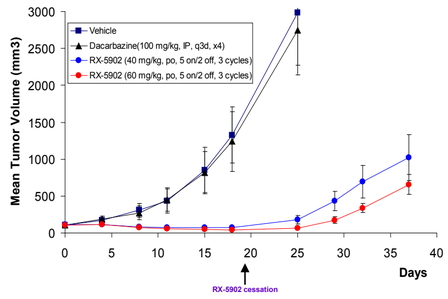


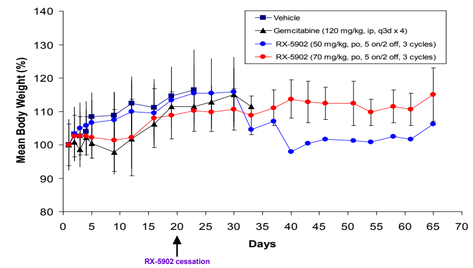
Inhibition of tumor growth by RX-5902

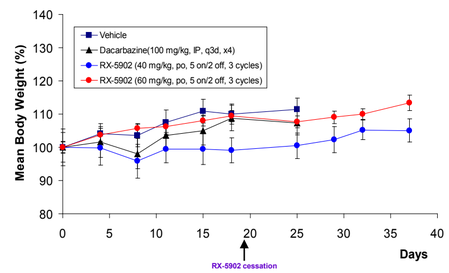
Human pancreatic cancer (MiaPaCa-2) xenograft



Human melanoma cancer (A375) xenograft

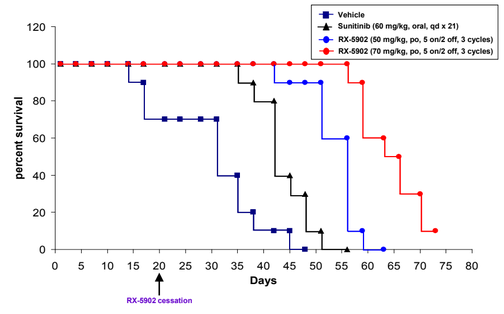




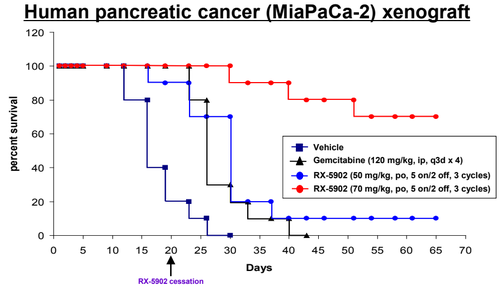


Survival benefit by RX-5902

Human renal cancer (Caki-1) xenograft



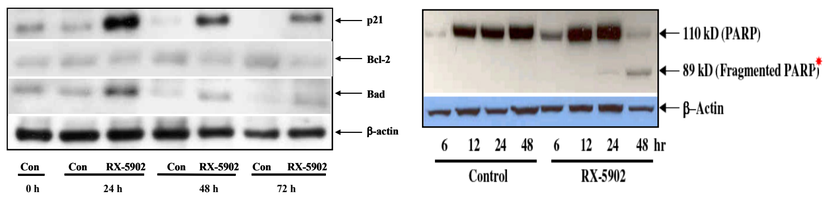
Human pancreatic cancer (MiaPaCa-2) xenograft

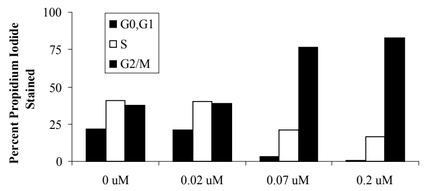


Pharmacokinetics

	Sprague-Dawley rats				Beagle dogs			
	Male		Female		Male		Female	
	IV	PO	IV	PO	IV	PO	IV	PO
Dose (mg/kg)	5	50	5	50	2	10	2	10
$AUC_{0\text{-inf}}$ (hr.ng/ml)	12652	39028	18566	107821	7426	14837	6642	30007
C_{max} (ng/ml)		1922		2695		1715		3710
$T_{1/2}$ (hr)	3.4	7.1	5.9	26.6	6.6	5.8	5.7	7.3
F (%)		30.8		58.1		53.7		97.1

Effect on apoptosis and cell cycle by RX-5902





Conclusion/Discussion

RX-5902:

- **inhibits the proliferation of human cancer cells at nanomolar concentrations.**
- **inhibits growth of drug-resistant cancer cells.**
- **inhibits/arrests tumor growth in melanoma and pancreatic xenograft models.**
- **significantly increases survival in renal and pancreatic xenograft models.**
- **shows high oral bioavailability in animal PK studies.**
- **may induce apoptosis and act against RNA helicase (data not shown).**

Ref: *Bioorg Med Chem. 2010 Nov 15;18(22):7966-74*

For further information about RX-5902 and Rexahn Pharmaceuticals, Inc., please contact Dr. DJ Kim: kimdj@rexahn.com, (240) 268-5306