Structure Guided Design, Synthesis, and Biological Evaluation of Oxetane-Containing Indole Analogues

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Supplemental Data Table of Contents

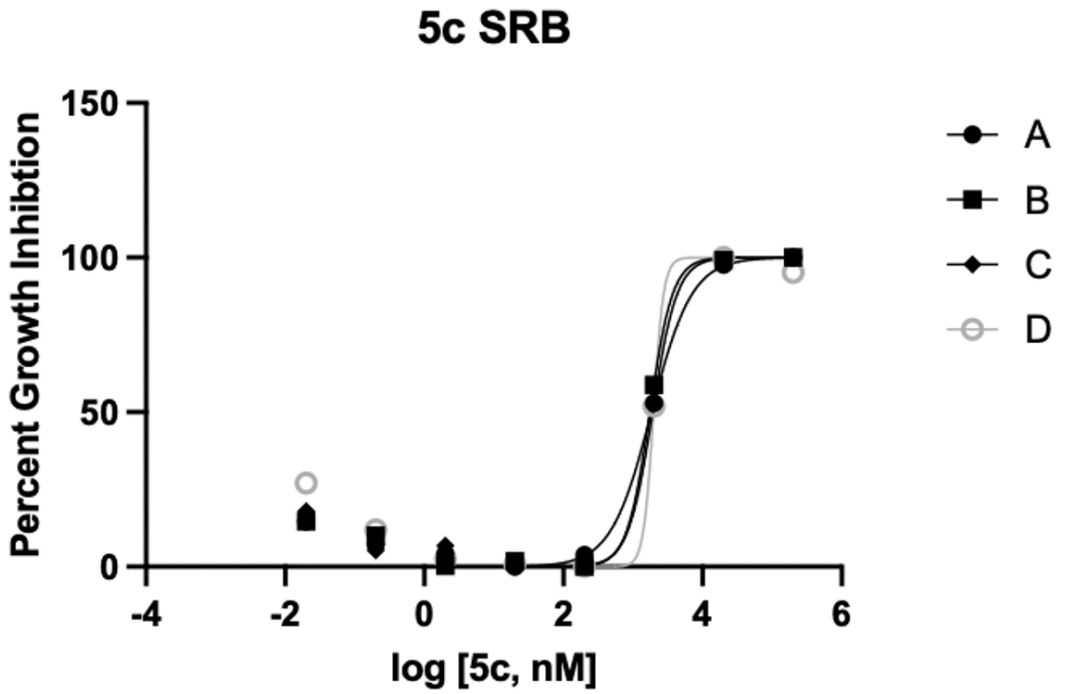
Cytotoxicity Assays (SRB)

SRB assays were performed in triplicates per experiment and in three independent experiments for each compound using 96-well plates plated at 12000 cells/well. Cells were allowed to adhere and grow for 24 h, then fixed with TCA as a 24 h growth control (day 0 control). On a separate 96-well plate, after 24 h cells were treated with various concentrations of drug for 48 h, and the same volume of growth media was added for the 72 h growth control (negative control). After a total time of 72 h, these plates were fixed with TCA. A Varioskan multimode plate reader was used to obtain the absorbance at 530 nm (OD values) for compound-treated samples, the day 0 control, and the 72 h negative control. Since experiments were performed in triplicate, the average reading of three columns was used to calculate the percentage of control cell growth and percent growth inhibition using the equation below[1]. GI_{50} values were obtained by transforming and normalizing the concentration vs growth inhibition plot and converting those data to a dose-response curve using GraphPad.

$$\%\ \text{of control cell growth} = \frac{\text{mean OD}_{\text{sample}} - \text{mean OD}_{\text{day 0}}}{\text{mean OD}_{\text{neg control}} - \text{mean OD}_{\text{day 0}}} \times 100$$

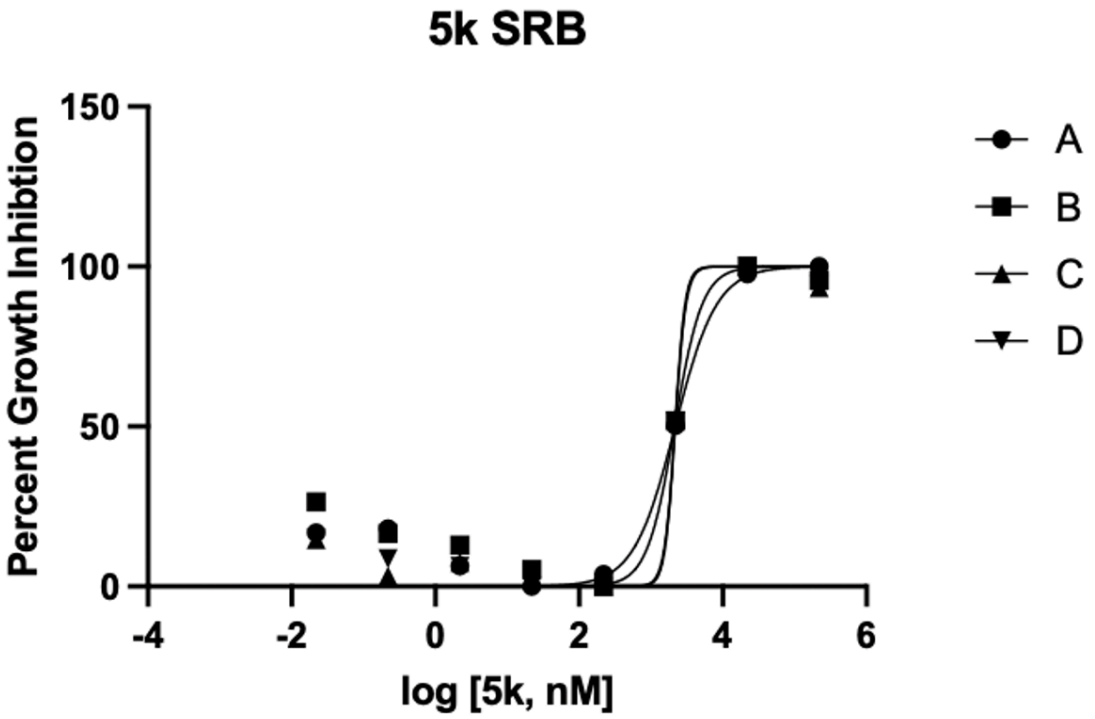
$$\%\ \text{growth inhibition} = 100 - \%\ \text{of control cell growth}$$

SRB in MDA-MB-231 (Human Breast Cancer) Cells



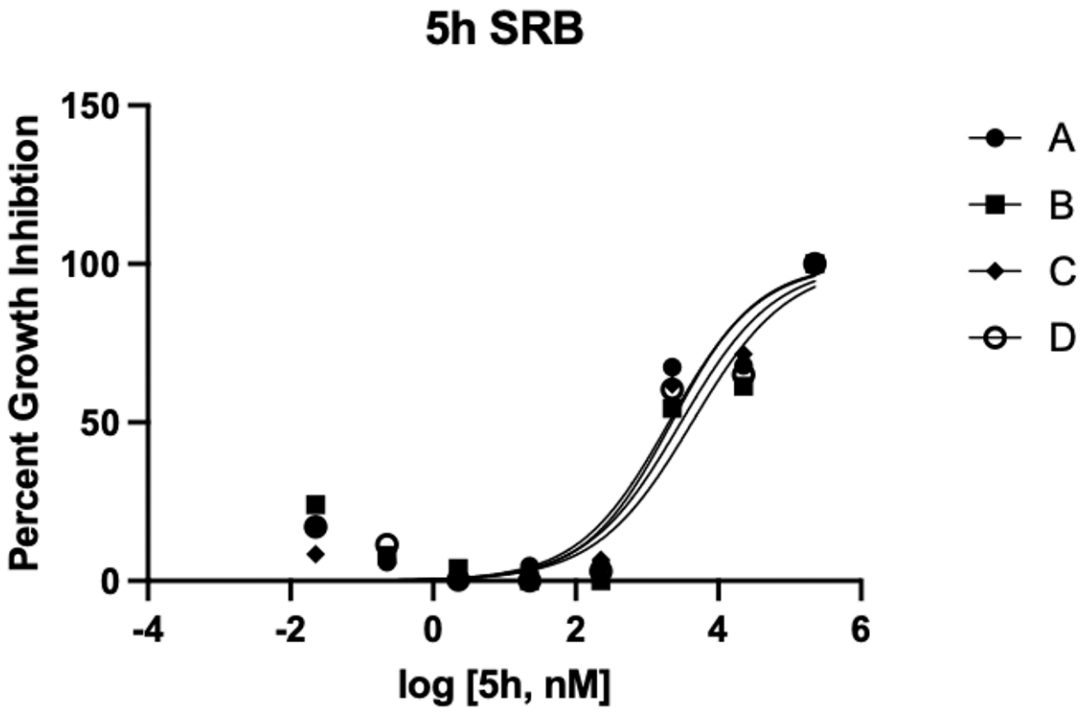
5c, nM	A	B	C	D
IC_{50}	1843	1747	1913	1972

Figure S1. Growth Inhibition (SRB) of MDA-MB-231 Cells Treated with 5c



5k, nM	A	B	C	D
IC_{50}	2176	2172	2165	2137

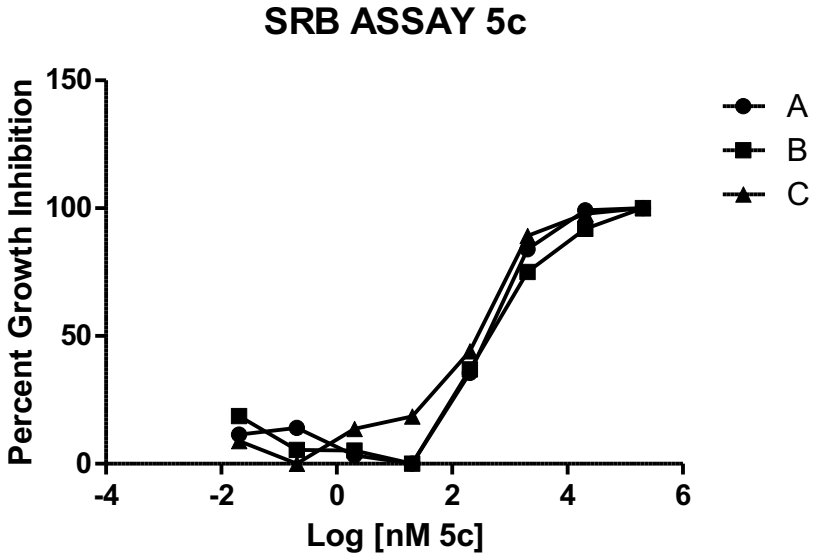
Figure S2: Growth Inhibition (SRB) of MDA-MB-231 Cells Treated with 5k



5h, nM	A	B	C	D
IC_{50}	2026	4285	2210	2981

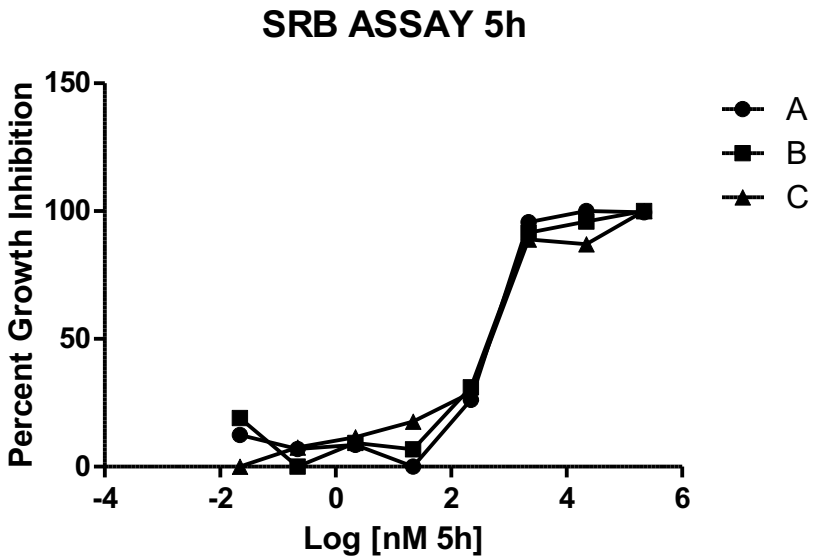
Figure S3. Growth Inhibition (SRB) of MDA-MB-231 Cells Treated with 5h

SRB in PANC-1 (Human Pancreatic Cancer) Cells



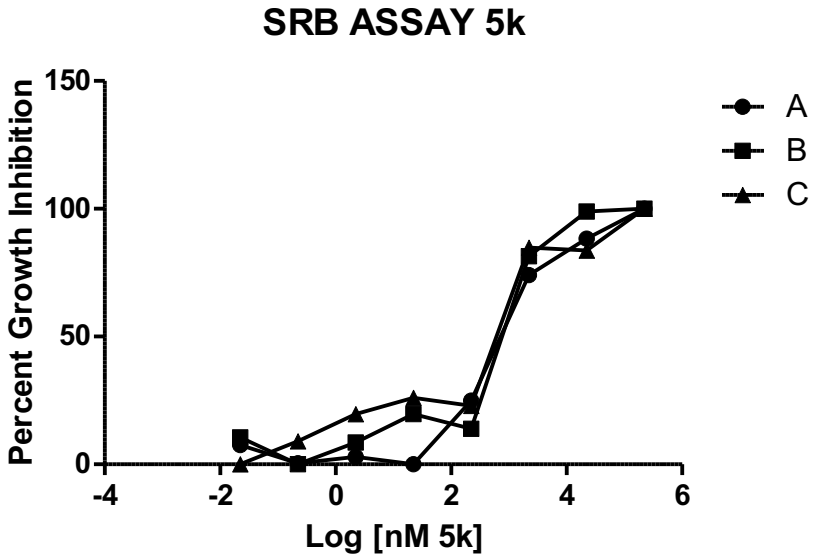
	A	B	C
IC_{50} (μM)	0.45	0.53	0.3

Figure S4. Growth Inhibition (SRB) of PANC-1 Cells Treated with 5c



	A	B	C
IC_{50} (μM)	0.43	0.43	0.45

Figure S5. Growth Inhibition (SRB) of PANC-1 Cells Treated with 5h



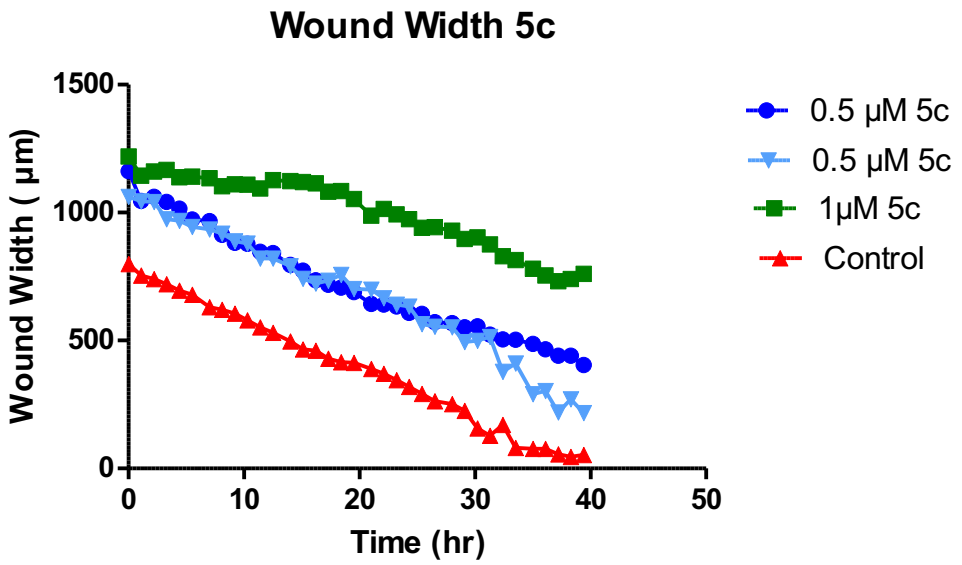
	A	B	C
IC_{50} (μM)	1.6	1.1	0.7

Figure S6. Growth Inhibition (SRB) of PANC-1 Cells Treated with 5k

Scratch Assay (Videos as movie format)

PANC-1 cells (ATCC CRL-1469) were cultured in T75 (Corning U420720U) flasks with Dulbecco's modified eagle media (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS) and 30 µg/mL gentamicin sulfate. The cells were grown in a humidified incubator supplemented with 5% CO_2 at 37 ℃. Cells were detached with Tryple, plated at a seeding density of approximately 1 x 10^5 cells in a 6-well plate and allowed to reach 70-80% confluency before treatment. A scratch was made with a sterile 10 µL pipette tip in each well before removing the media and any detached cells. Two wells of PANC-1 cells were treated with either 1 µM or 0.5 µM of **5c** or **5h**, with two wells as controls with a final DMSO content of less than 0.2% and a final media volume of 3 mL in each well.

The wound closure was monitored with an automated Biotek Lionheart ELx800 microscope with a 4X objective for 48 h. We utilized the supplemented Gen5 software to perform a cellular and statistical analysis on the wound closure data.



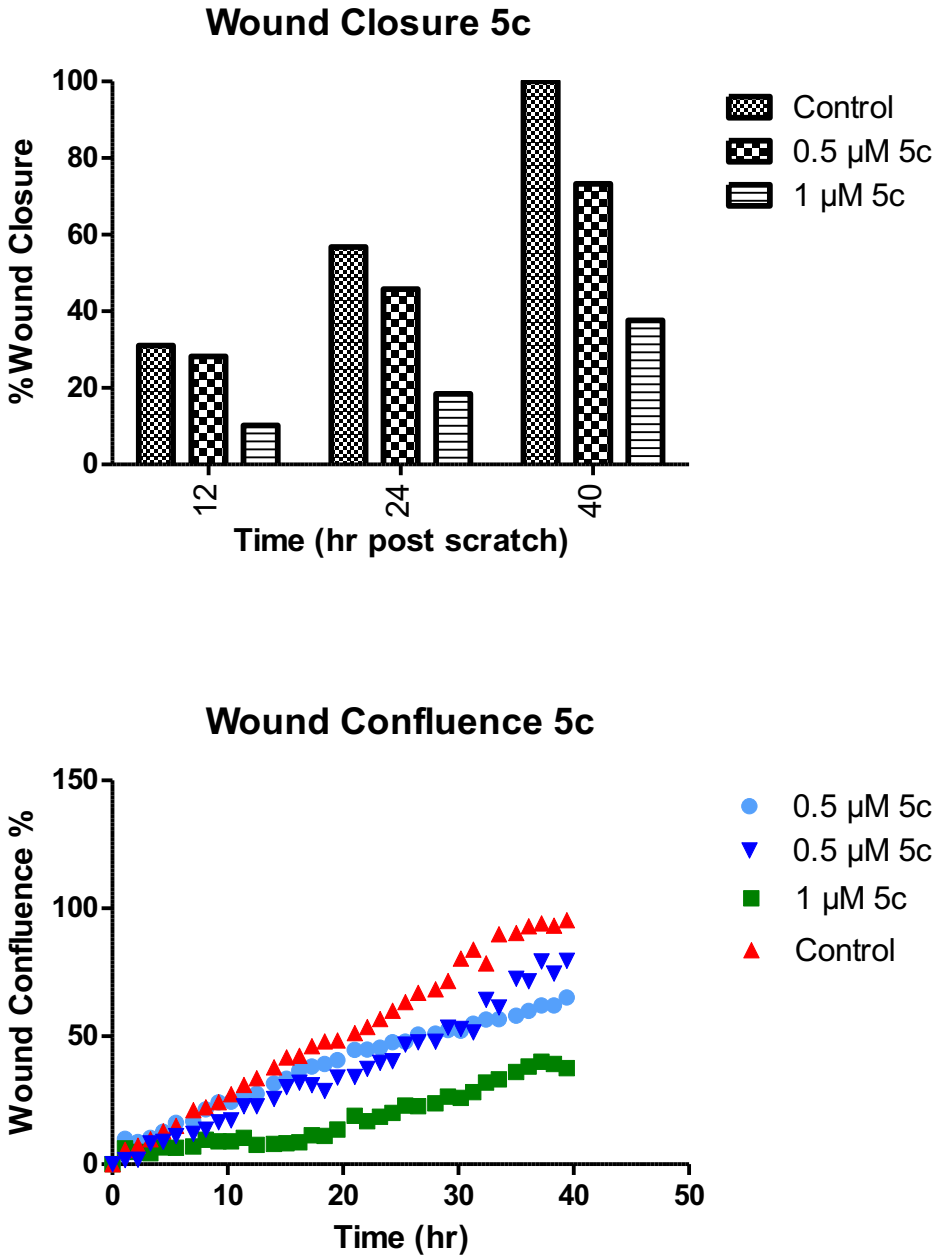
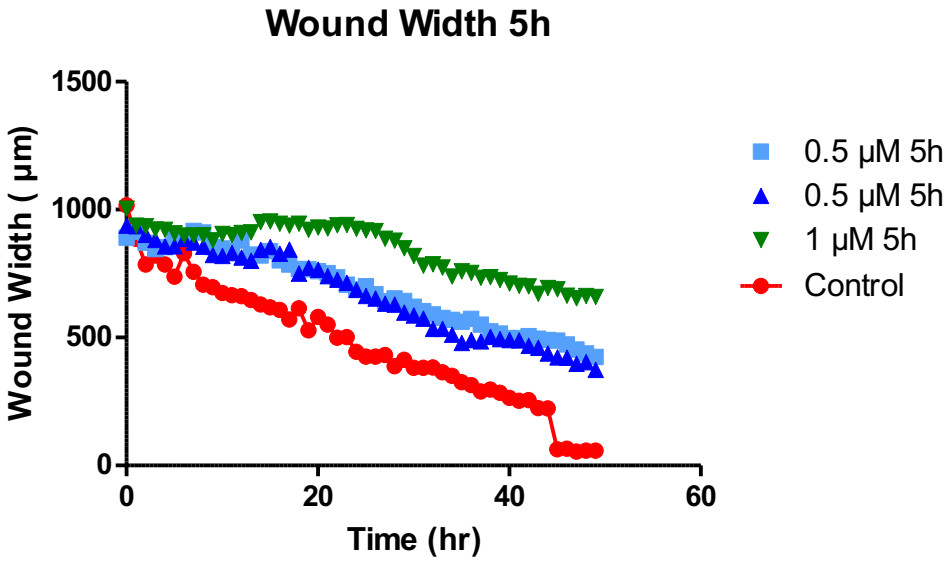
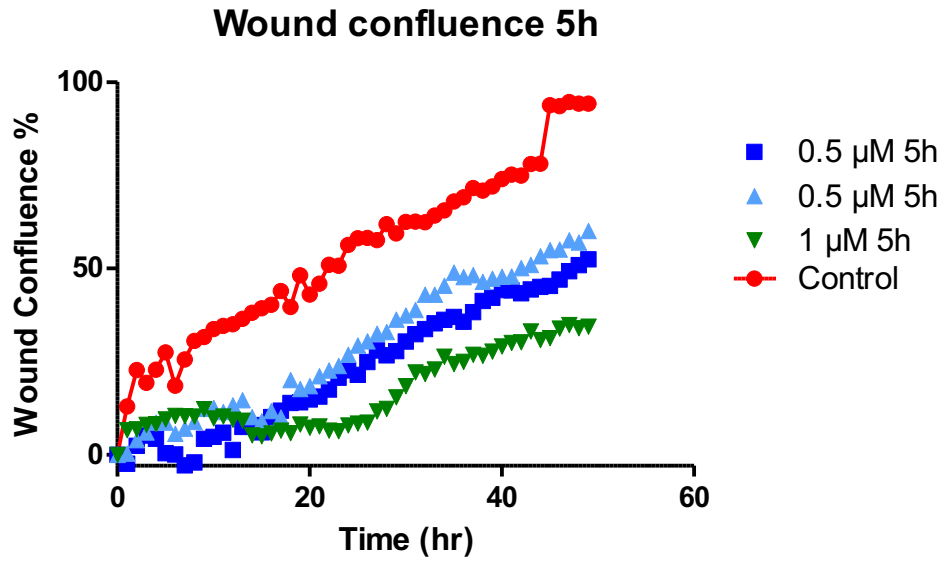


Figure S7. Wound Width, Closure and Confluence in PANC-1 Cells Treated with 5c


Wound Width 5h
Wound Width (µm)
1500
1000
500
0
0
20
40
60
Time (hr)
0.5 µM 5h
0.5 µM 5h
1 µM 5h
Control


Wound confluence 5h
Wound Confluence %
100
50
0
0
20
40
60
Time (hr)
0.5 µM 5h
0.5 µM 5h
1 µM 5h
Control

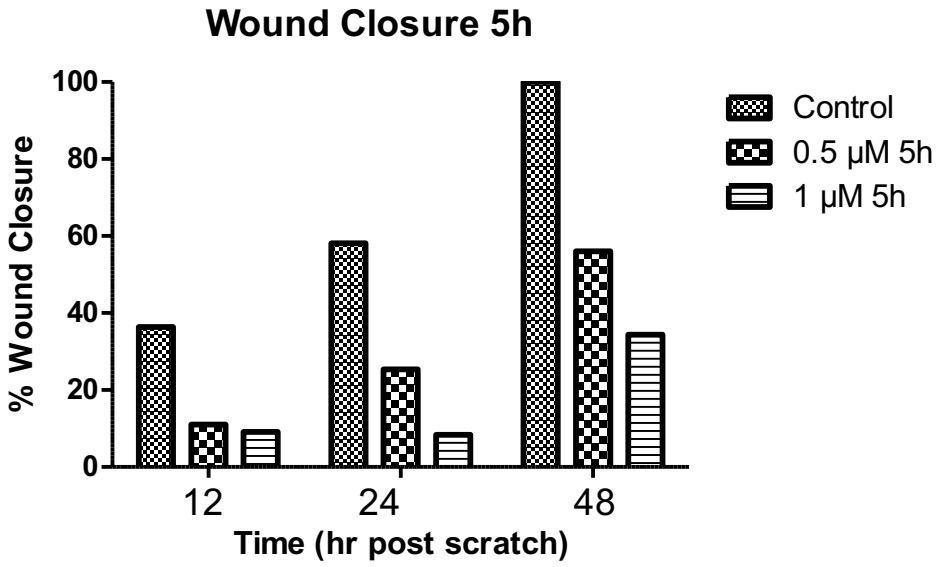


Figure S8. Wound Width, Closure and Confluence in PANC-1 Cells Treated with 5h

References:

(1) Vichai, V.; Kirtikara, K. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nature Protocols* **2006**, *1* (3), 1112-1116. DOI: 10.1038/nprot.2006.179.