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Confidential

QL13 QL13-01

Done

Title: QL13-01

Goal: PTO Cy5 uptake, 500 nM, 1 h preincubation + 2 h incubation with different inhibitors, two plates, activation

Started on: March 10, 2021

Modified on: June 9, 2021

Closed date

Project: Phosphorothioates

Type: PTO Cy5 Inhibition

HeLa Kyoto cells were seeded at 8×10^4 cells/mL in FluoroBrite DMEM + 10% FBS on μ -Plates 96-well ibiTreat sterile and kept at 37 °C with 5% CO₂ overnight. The following day, cells were washed with FluoroBrite DMEM (3 × 3 mL/well) using a plate washer, keeping a final volume of 135 μ L/well.

Dilutions of Cy5-labelled phosphorothioate and inhibitor in PBS were prepared in a 96-well V-bottom plate and added to the μ -Plate containing the cells at the desired time (15 μ L/well, 10x final concentration in PBS) to reach a final volume of 150 μ L/well, and cells were incubated for 1 h at 37 °C with 5% CO₂ in DMEM. Cells were washed with FluoroBrite DMEM (3 × 3 mL/well) using a plate washer, keeping a final volume of 135 μ L/well, and the corresponding solution of phosphorothioate was added (15 μ L/well, 10x final concentration in PBS) to reach a final volume of 150 μ L/well, and cells were incubated for 2 h at 37 °C with 5% CO₂ in DMEM.

Then, Hoechst 33342 (15 μ L/well, 100 μ g/mL in PBS) was added. After 15 min of incubation at 37 °C with 5% CO₂, the medium was removed and cells were fixed with a PFA solution (3% in PBS, 80 μ L/well) for 20 min. The plate was washed with PBS (3 x 3 mL/well) and imaged using a high-content automated microscope.

PLATE 1

Sequence: AGGTCCCCATACACCGAC

1 h pre-incubation, 2 h incubation, 37 °C

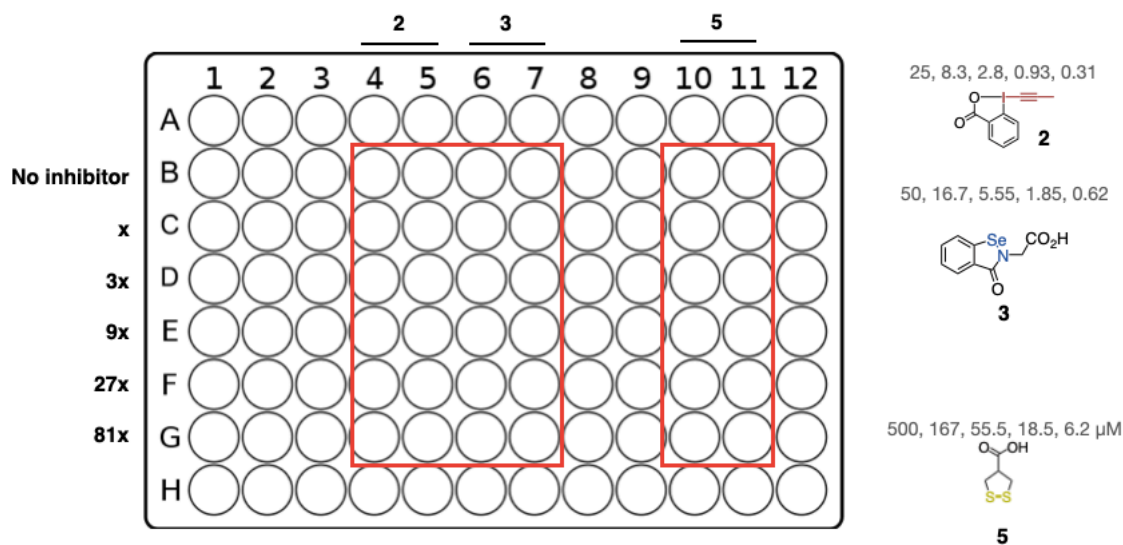
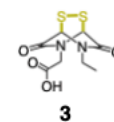


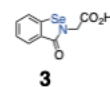
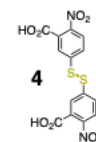
PLATE 2

Inhibitor

	Inhibitor					Activation						
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												



100, 33.3, 11.1, 3.7, 1.23

Activation20 x = 500 μ M
Final = 25 μ M20x = 1 mM
Final = 50 μ M20x = 10 mM
Final = 500 μ M**Attached files**

QL13-01-Activation.pzfx
 QL13-01-Plates.key
 QL13-01-Inhibition.pzfx