Screen Targeting Lung and Prostate Cancer Oncogene Identifies Novel Inhibitors of RGS17 and Problematic Chemical Substructures

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Table SI: Screening results of NCI NExT Diversity Library

NCI library	60,502 compounds	hit rate
initial hits	131 compounds	0.22%
non PAINS	110 compounds	0.18%
3 point biotin-GST counter screen	36 compounds	0.06%
protein-protein interaction dose response	16 compounds	0.03%
biotin-GST dose response (most selective)	5 compounds	0.01%

Table SII - Results of SMARTS RDKit PAINS filtration with explicit hydrogens merged to query filter. Matching (over, equivalent, under, none) are with respect to results by Saubern et al. for the original SLN filters. Filter names are from reference 52. Black, un-bracketed numbers are our results. Red (numbers) represent results by Saubern et al. for RDKit using a 3-pass filtration process. Red [numbers] represent results by Saubern et al. for RDKit, no 3-pass filtration.

	over match	equivalent	under match	no match
p_m150	1 (0)	11 (4)	4 (5)	0 (7)
p_l150	12 (1)	27 (14)	3 (2)	11 (36)
p_l15	2 (14)	77 (3)	1 (1)	10 (72)
total	15 (15) [7]	115 (21) [31]	8 (8) [7]	21 (115) [115]

Table SIII - Calculated IC_{50} values for the curves in Figure S2. Control data not shown. Hits were selected as leads based on RGS17 IC_{50} value results. NC = not converged, UC = un-calculable.

compound	RGS17 IC_{50} (µM)	control IC_{50} (µM)
SAM003813654	43.7	>100
SAM003765252	35.0	>100
SAM003821917	46.4	>100
SAM003697649	40.6	99.4
SAM003814748 (IV)	31.1	NC
SAM003813578	41.8	>100
SAM003823278 (I)	9.55	>100
SAM003792154 (II)	19.2	>100
SAM003697650	44.0	>100
SAM003692151 (V)	14.3	>100
SAM003765221	48.2	>100
SAM003692405	49.7	>100
SAM003692400	49.2	>100
SAM003761383	39.5	NC
SAM003792514 (III)	14.4	>100
SAM003692403	50.0	>100

Table SIV – IC_{50} values calculated for the curves depicted in Figure 3. Values are represented as the 95% CI IC_{50} value in µM.

compound	initial from library	t = 0	t = 7 days	t = 14 days	t = 28 days	b-GST control t = 14 days
I	4.57 to 17.2	> 100	24.6 to 63.5	5.41 to 15.3	1.26 to 10.2	> 100
IV	21.4 to 44.4	> 100	10.6 to 14.7	5.22 to 9.87	3.50 to 14.9	> 100
V	9.28 to 21.4	NC	5.06 to 7.55	2.07 to 3.76	1.83 to 4.89	> 100

Table SV – IC$_{50}$ values for the curves in Figures 5A, 5B, reported at the 95% CI.

compound	IC$_{50}$ µM 95% CI
II	12.2 to 29.2
II-1	62.2 to > 100
II-2	40.6 to 97.6
II-3	3.10 to 9.12
II-4	NC
II-5	NC
II-6	> 100
II-7	> 100
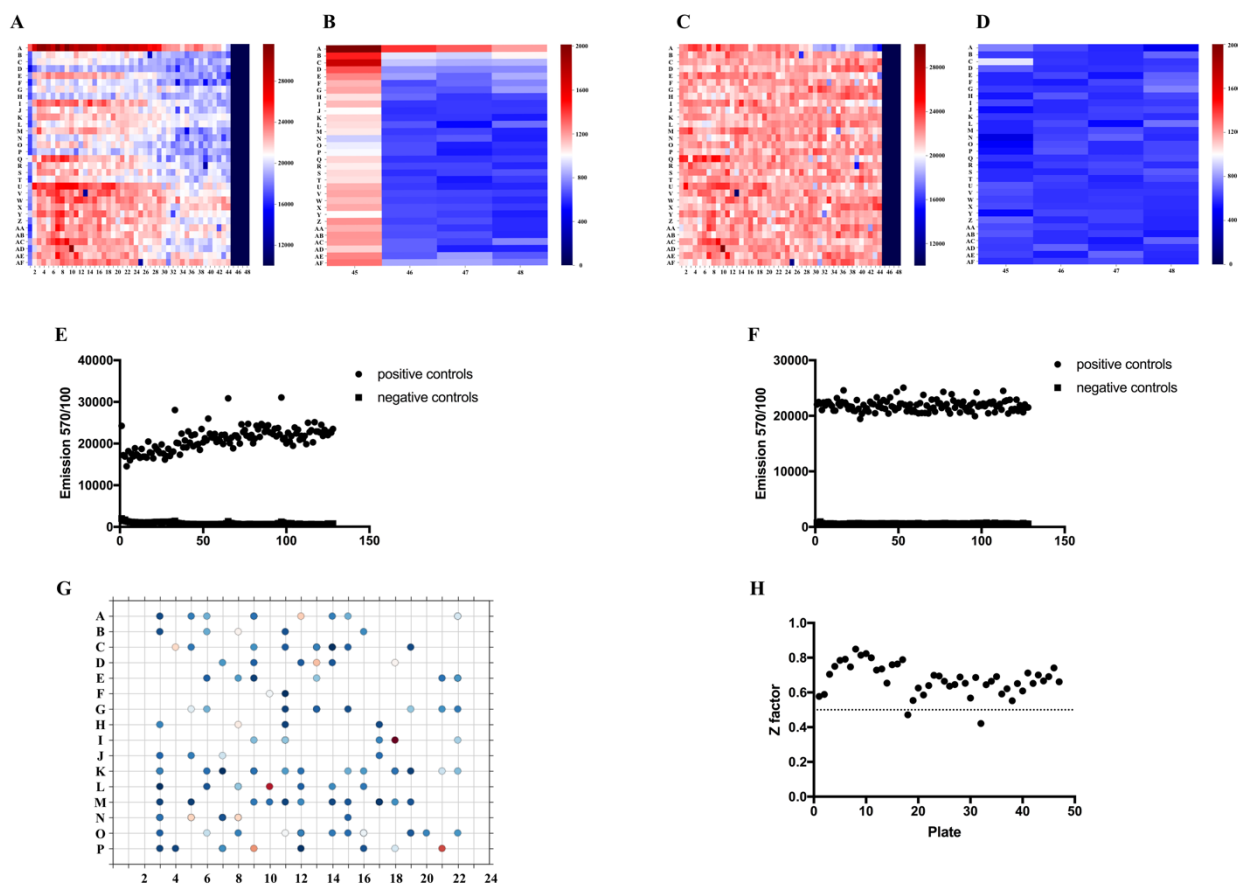
Figure S1 – A/B.) Heatmap of sample screening data for one 1536 well plate, A represents the whole plate where B represents a closer look at the negative control wells. Edge effects and row biases are noted in the uncorrected plate data. C/D.) Same plate data as A and B post B-score analysis, see methods. E.) Positive and negative controls from A. F.) Positive and negative controls of the B-score corrected data from C. G.) Use of B-score correction did not introduce plate bias – hit map of all 131 identified hits show a relatively equal distribution. Hits projected onto a 384 well plate corresponding to the format of the parent plates. H.) Z-factor for each plate in the screen, each containing 128 internal positive and 128 internal negative controls. Z factors are on B-score corrected data.
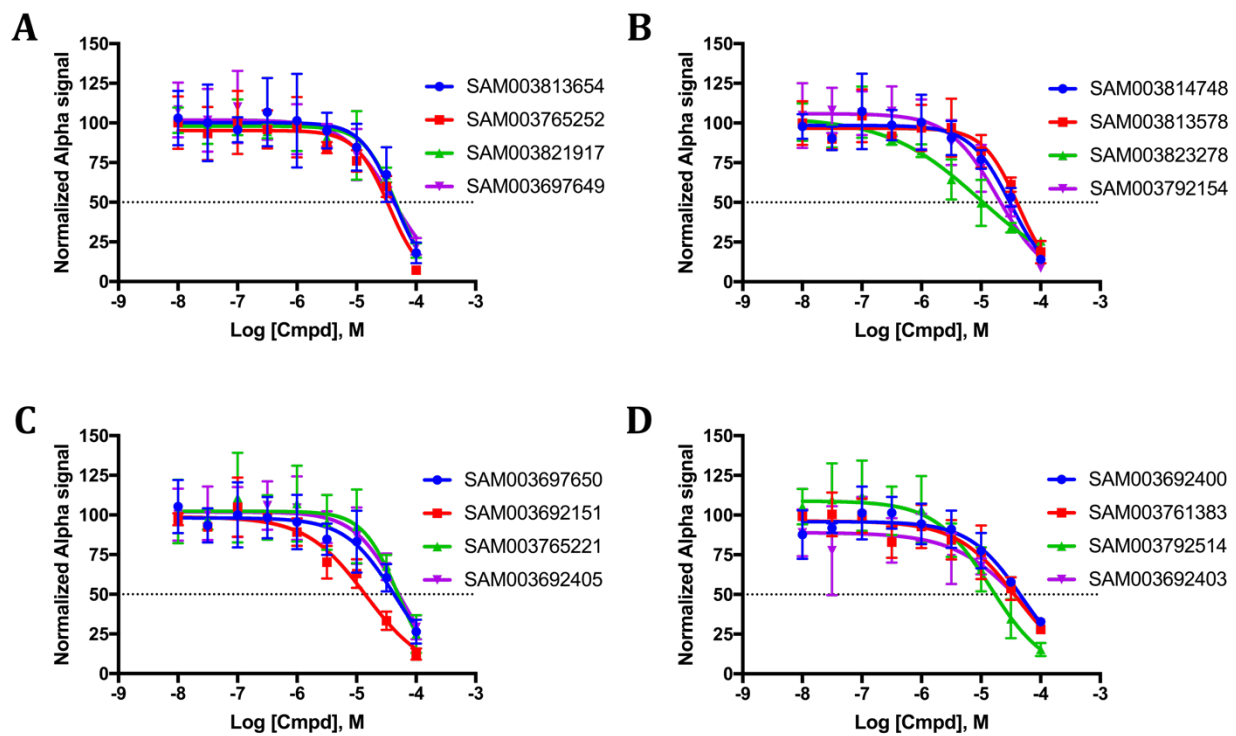
Figure S2 – Dose response analysis of the 16 hit compounds that were tested in n=3. IC_{50} values are listed in table SII. Data are n=3 in duplicate shown as mean ± SD.
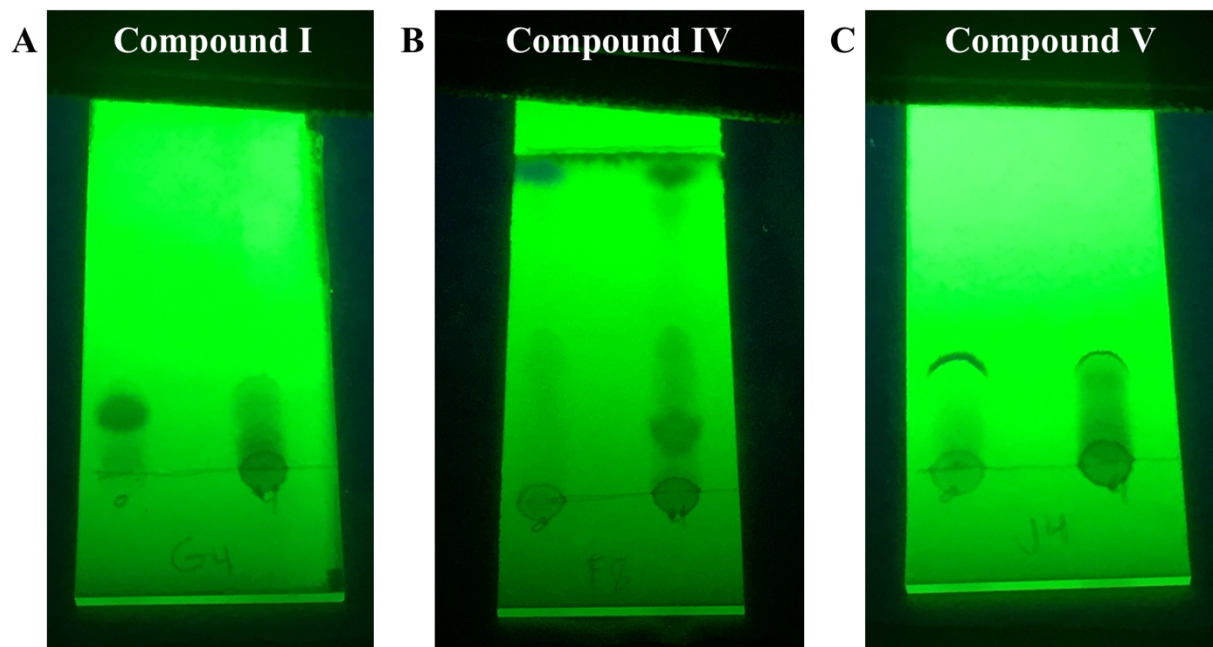
Figure S3 – TLC analysis of lead compounds found to exhibit increased potency when incubated at RT for an extended time. For each image, freshly made compound is at left corresponding to t = 0 data from Figure 3, and t = 28 days product is at right. For each compound, t = 28 days products display a different pattern from t = 0 compound, indicating decomposition of the parent compound.

A
Compound I
t=0

B
Compound I
t=7 days

C
Compound I
t=14 days

D
Compound I
t=28 days

E
Compound IV
t=0

F
Compound IV
t=7 days

G
Compound IV
t=14 days

H
Compound IV
t=28 days

I
Compound V
t=0

J
Compound V
t=7 days

K
Compound V
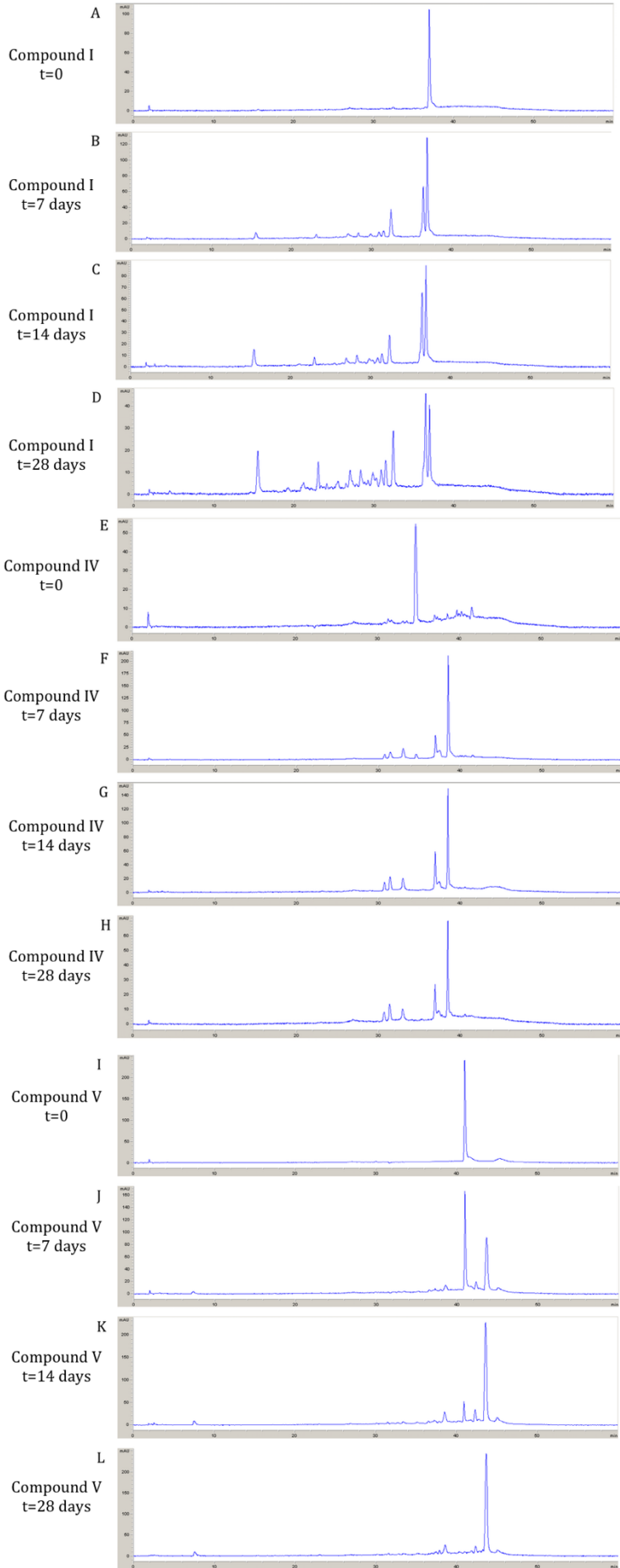t=14 days

L
Compound V
t=28 days

Figure S4 – HPLC analysis of compound I (A-D), IV (E-H), and V (I-L) degradation products.