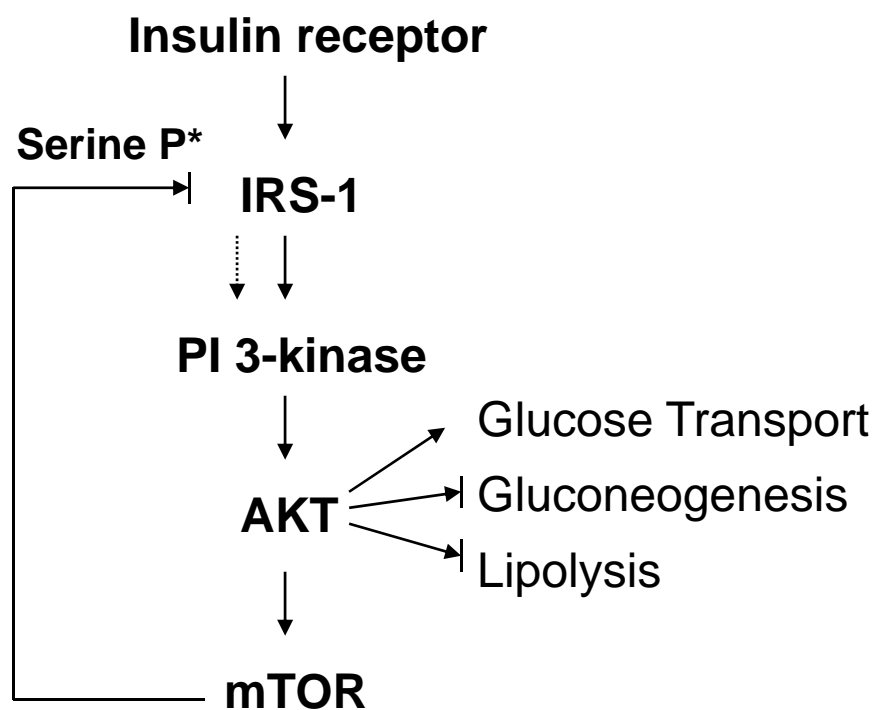


Pharmacological Inhibition of a Kinase involved in Insulin Signaling

**Siobhan Malany, Ph.D.
Tanabe Research Laboratories**

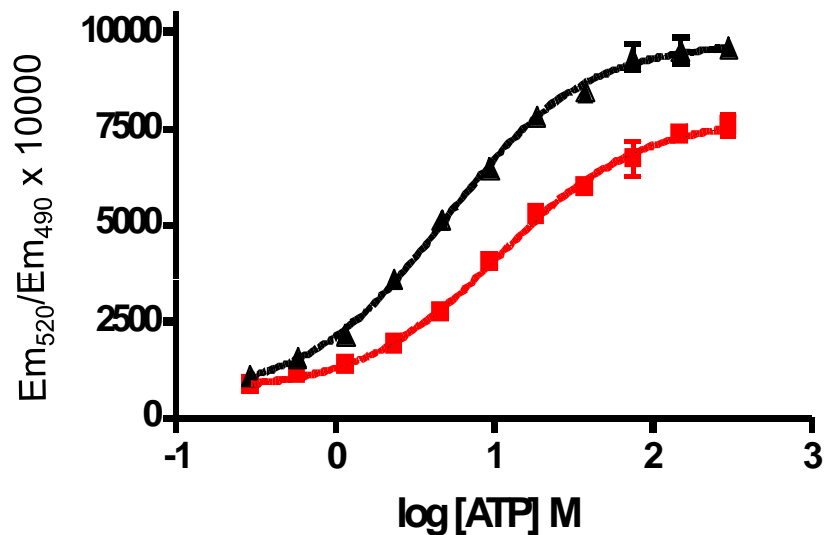
Negative Regulator of Insulin Signaling via Phosphorylation of IRS-1



In vitro compound profiling

- Primary enzyme screen (HTRF)
- Inhibition of substrate phosphorylation in cells
- Caliper mobility technology
 - Mechanism of action studies
 - Kinase selectivity profiling
- Concentration-dependent activity on insulin signaling

Homogeneous Time-Resolved FRET Assay to Measure Enzyme Activity



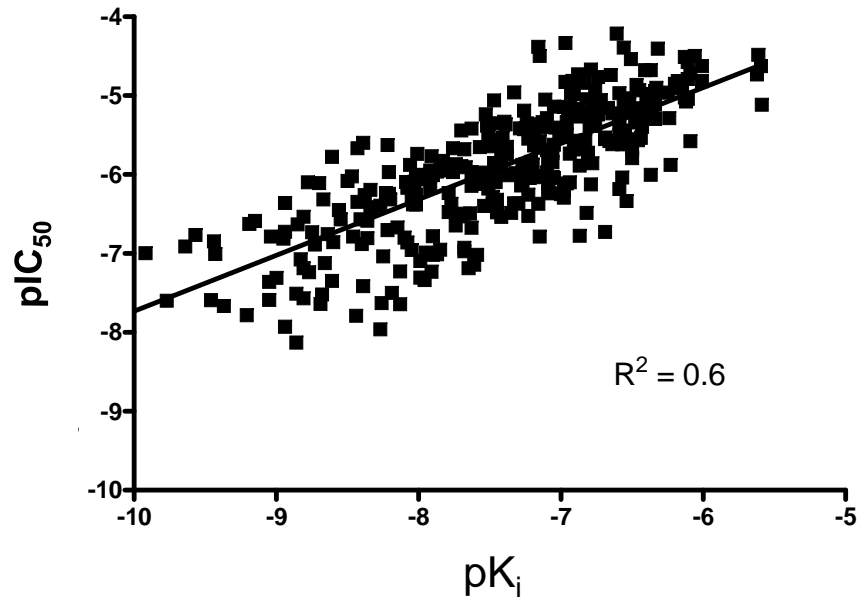
- Apparent ATP $K_m = 8\mu\text{M}$ of enzyme activation
- Reactions containing enzyme and ATP initiated by fluorescein-labeled peptide
- Reactions quenched by addition of EDTA/Tb-labeled antibody

Enzyme vs. Functional Assay Correlation

Biochemical Assay Optimization

- 384-well assay
- Serial dilution in 100% DMSO
- Linear Initial rates over 60 min
 - [ATP] up to 100uM
- Minimal [enzyme] = 2.5nM
- S/B = > 6-fold
- Z-factor = 0.7

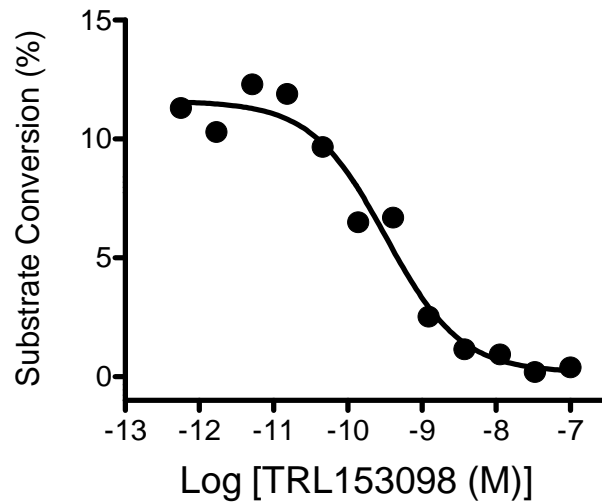
pK_i Vs. pIC₅₀



Lead Compound TRL153098

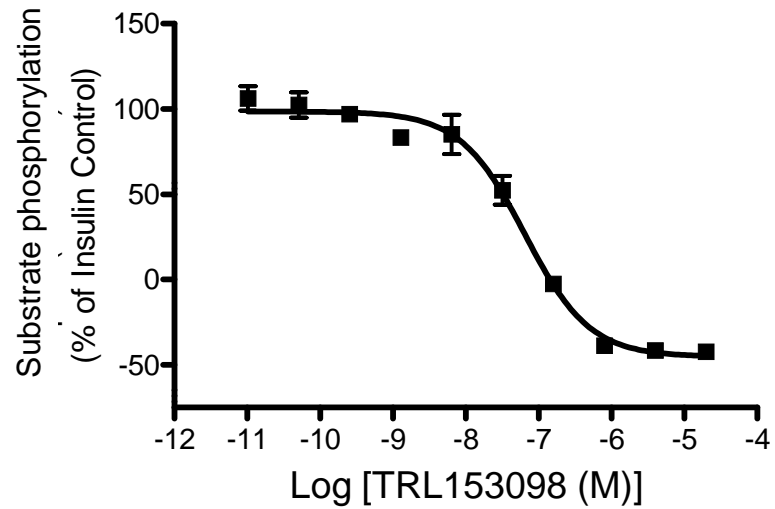
Potent Inhibitor of enzyme activity and cellular phosphorylation

HTRF Biochemical Assay



Apparent K_i value of 2.2

C2C12 insulin dependent phosphorylation Assay

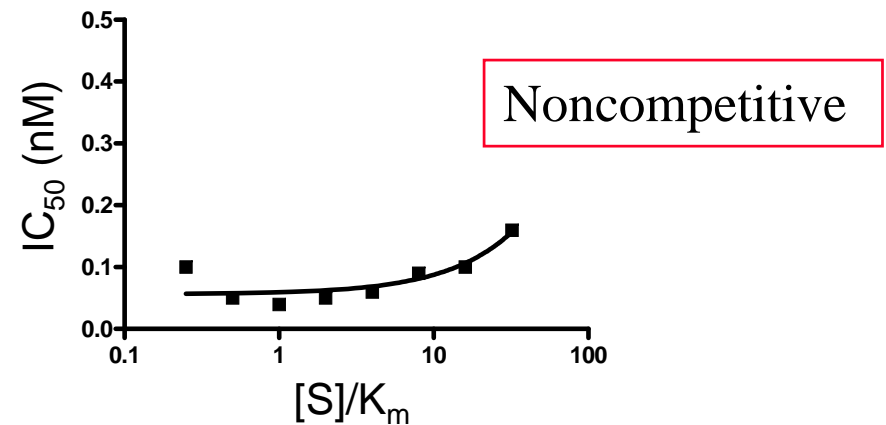
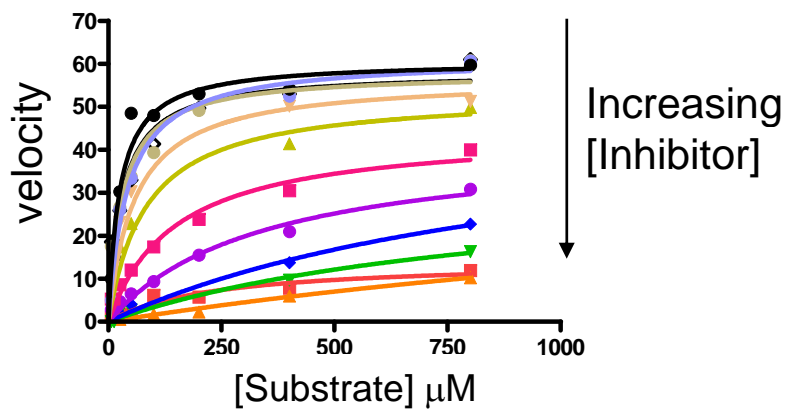
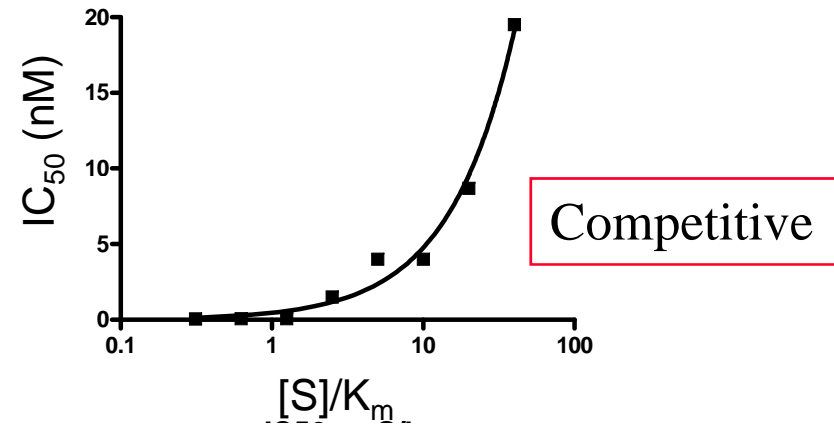
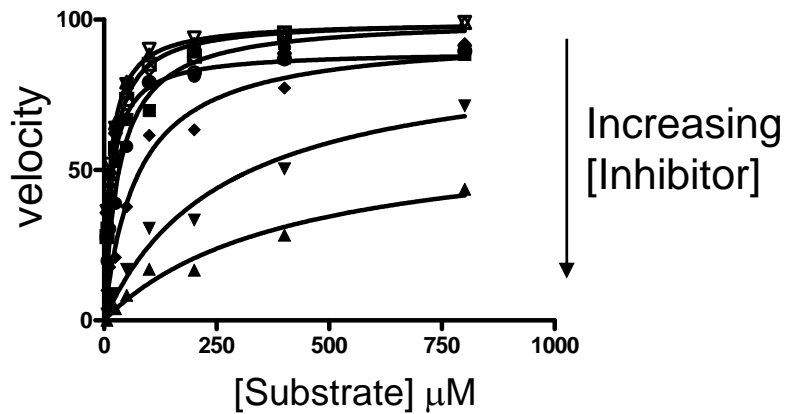


IC_{50} value of 56.3 nM

Caliper Mobility-Shift Enzyme Modality Studies

TRL153098 is ATP Competitive

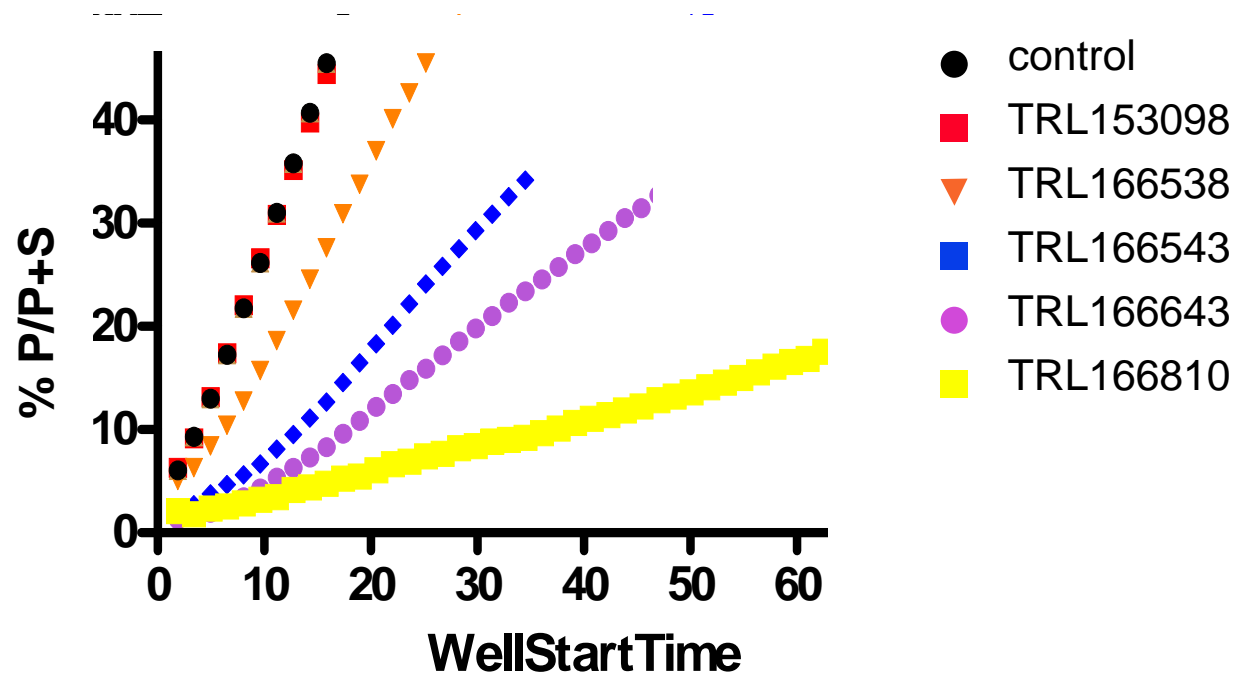
Steady-State Analysis of Inhibition Mechanism
Using Labchip EZ Reader 12-sipper



Caliper Mobility-Shift Enzyme Reversibility Studies

TRL153098 is reversible inhibitor

Recovery of enzyme activity after rapid dilution for reversible and slowly reversible inhibitors



- Inhibitor (10 X IC_{50}) and enzyme (100X) pre-incubated for 1 hour at RT.
- Pre-incubation mixture diluted 100-fold into saturating amounts of ATP (10 X K_{mapp}).
- The dissociation rates (k_{off}) determined by eq: $p = V_i * t + (v - v_i) * (1 - \exp^{-k_{off} * t}) / k_{off}$

Caliper ProfilerPro Kinase Selectivity Assays

TRL153098 selective inhibitor across 120 kinases

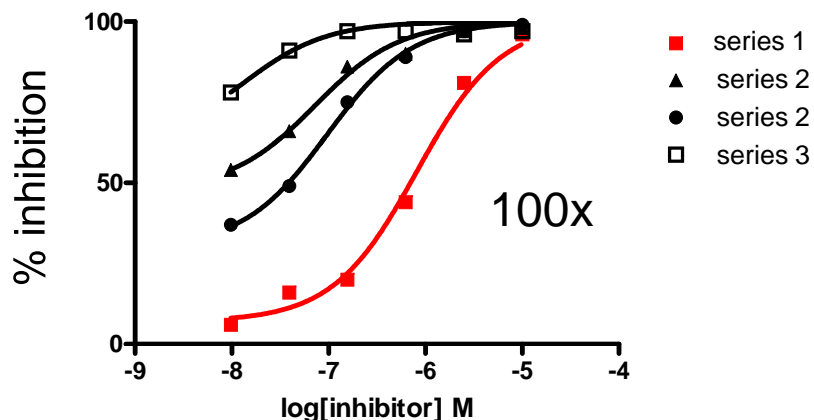
Percent Inhibition at 1 μ M

Kinase	Compounds			
	153098 series 1	167015 series 2	153021 series 2	167175 series 3
MAPKAPK2	0	0	12	0
AurA	33	47	22	18
PKCz	71	0	8	17
RSK1	48	88	66	97
PRAK	1	1	7	0
Erk1	2	6	4	10
PKD2	13	29	1	0
CK1d	47	64	4	0
CHK1	13	3	10	25
ABL	28	0	1	0
FYN	28	7	2	44
LYN	22	10	2	70
CHK2	22	32	3	46
MET	12	0	0	8
LCK	36	13	2	49
SRC	28	6	8	60
GSK3b	11	32	33	94
Erk2	4	5	4	12
PKA	73	98	76	91
AKT2	7	46	12	53
INSR	17	0	0	10
p38a	4	5	0	0
AKT1	17	90	42	94
MSK1	81	96	85	97

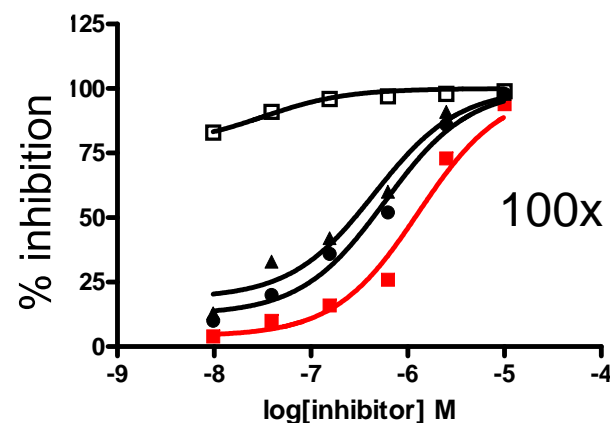
Caliper ProfilerPro Kinase Selectivity Assays

TRL153098 (red) showed superior selectivity profile

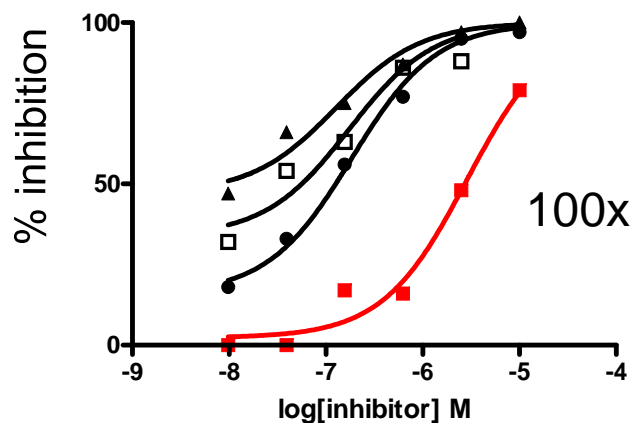
Mitogen and stress-activated Kinase 1 (MSK1)



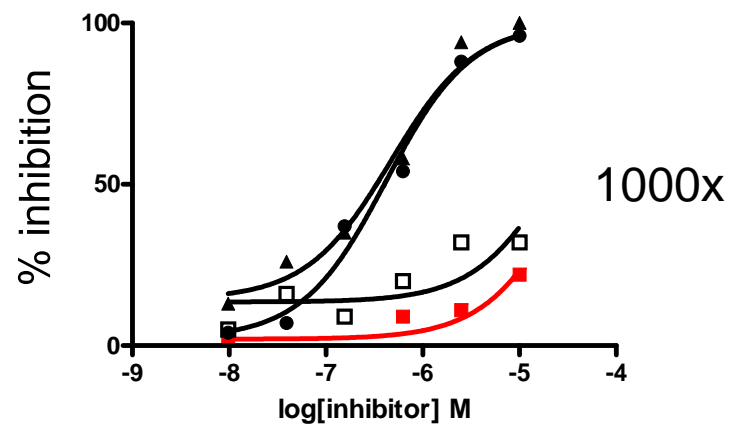
Protein Kinase A (PKA)



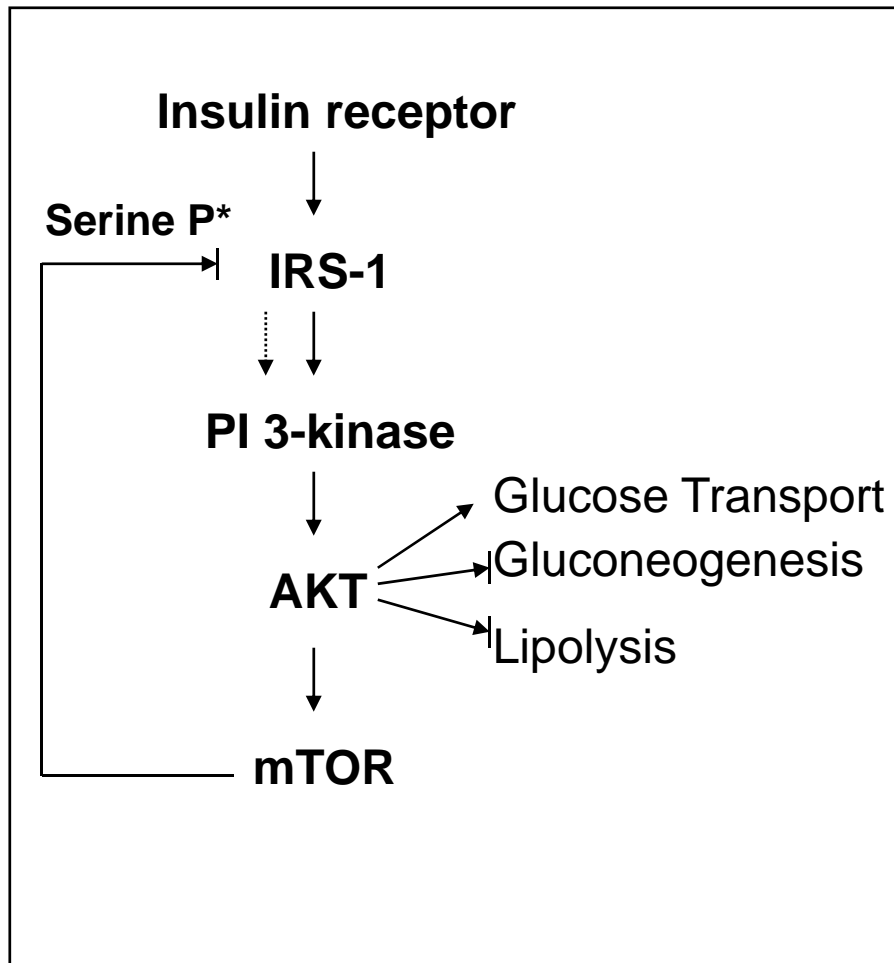
p90 ribosomal S6 kinase (RSK1)



Glycogen synthase kinase-3b (GSK3b)



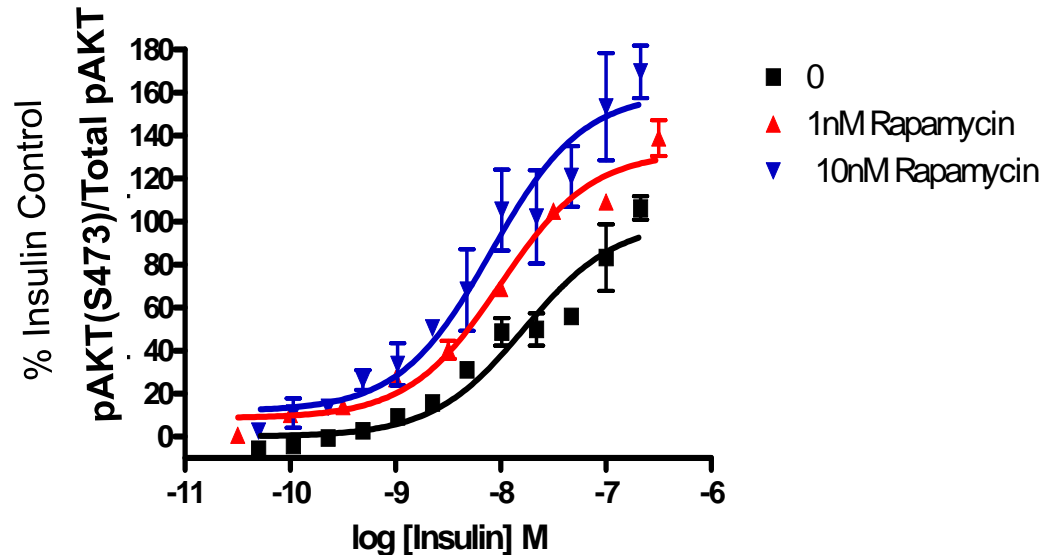
Negative Regulator of Insulin Signaling via Phosphorylation of IRS-1



- **Glucose uptake studies**
- **Phosphorylation of AKT**
- **Phosphorylation of IRS-1**
- **Cell Lines:**
 - C2C12 mouse myotubes
 - 3T3-L1 differentiated adiposites
 - HepG2
 - CHO-IR

Determination of Insulin dose-dependent pAKT Levels in 3T3-L1 Cells

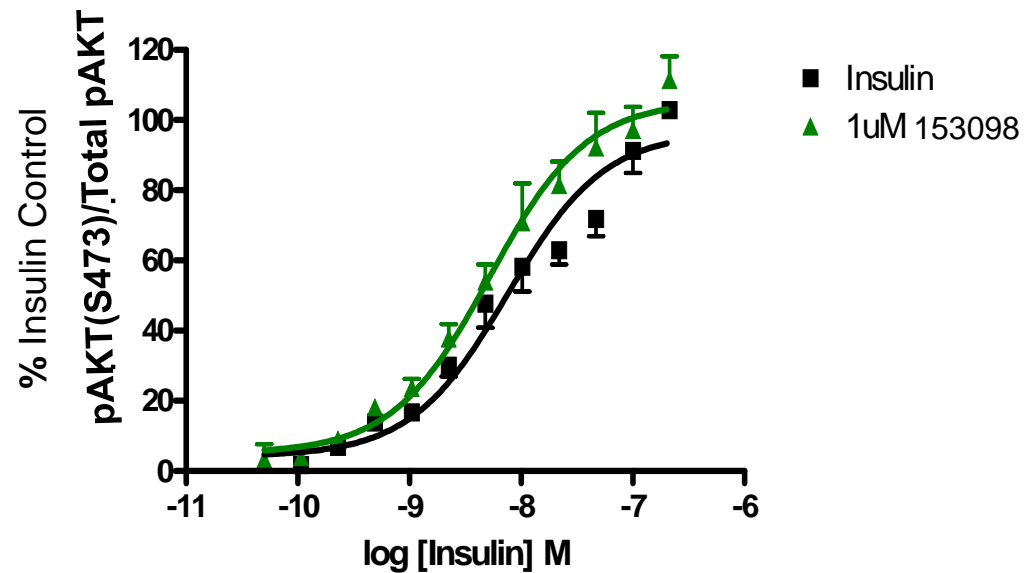
Average of 2 separate experiments



- Response to insulin alone $EC_{50}=10 \pm 2$ nM (n=5)
- Enhancement in pAKT levels at 10nM Rapamycin (57% at 100nM insulin)
- No Significant affect on EC_{50} value of insulin

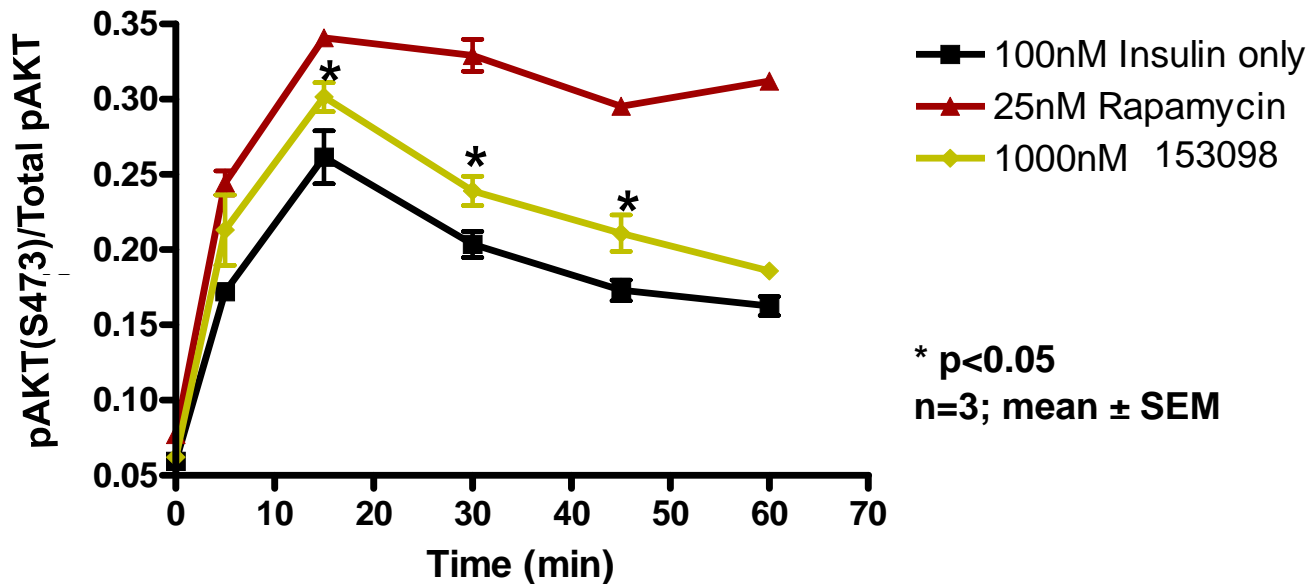
TRL153098 Minimally Enhances pAKT levels in 3T3-L1 Cells

Average of 4 separate experiments



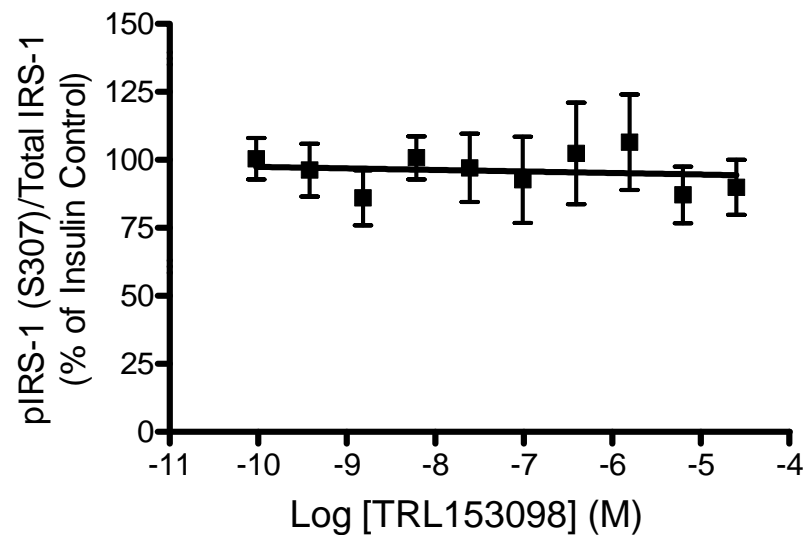
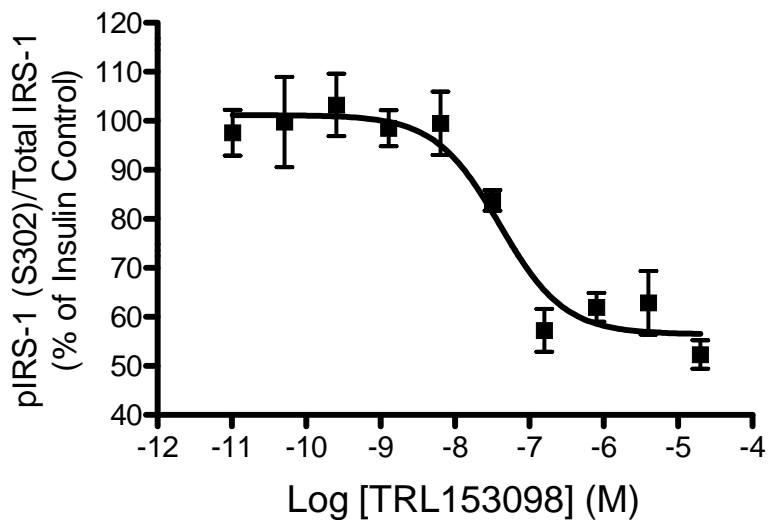
- Enhancement in pAKT levels at 1uM compound (25% at 100nM insulin)

TRL153098 Minimally Enhances pAKT levels HepG2 Cells

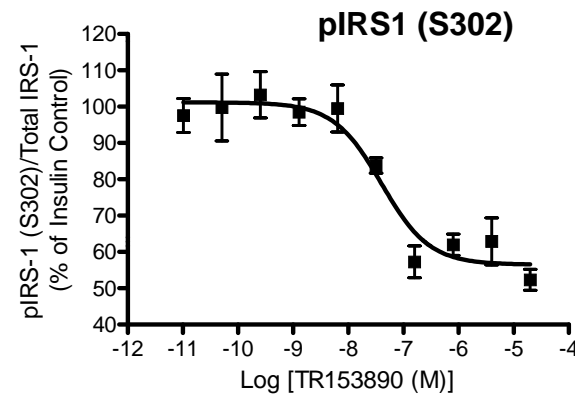
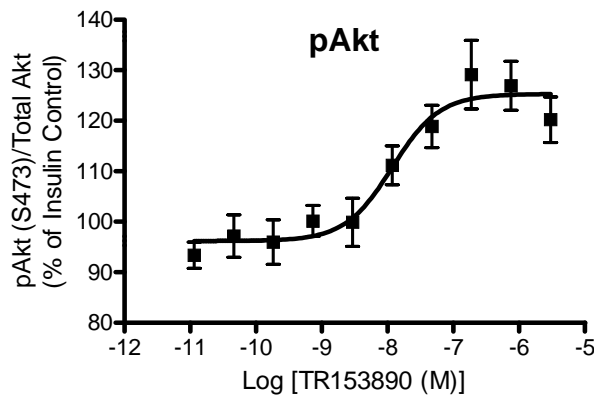
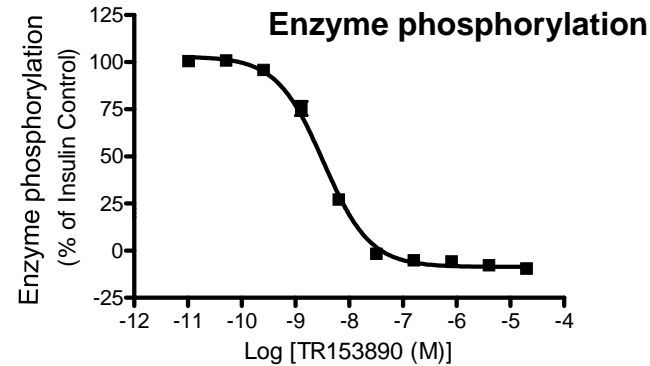
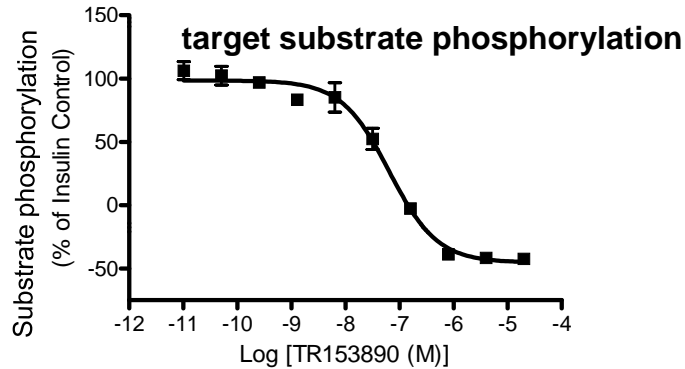


- Compound in insulin-treated cells showed significant increase (15-24%) in pAkt compared with vehicle at the 10, 30 and 45 min time points (* p<0.05)
- Rapamycin showed 30-70% increase over same time

TRL153098 Suppresses pIRS (S302) but not (S307) Levels CHO-IR Cells



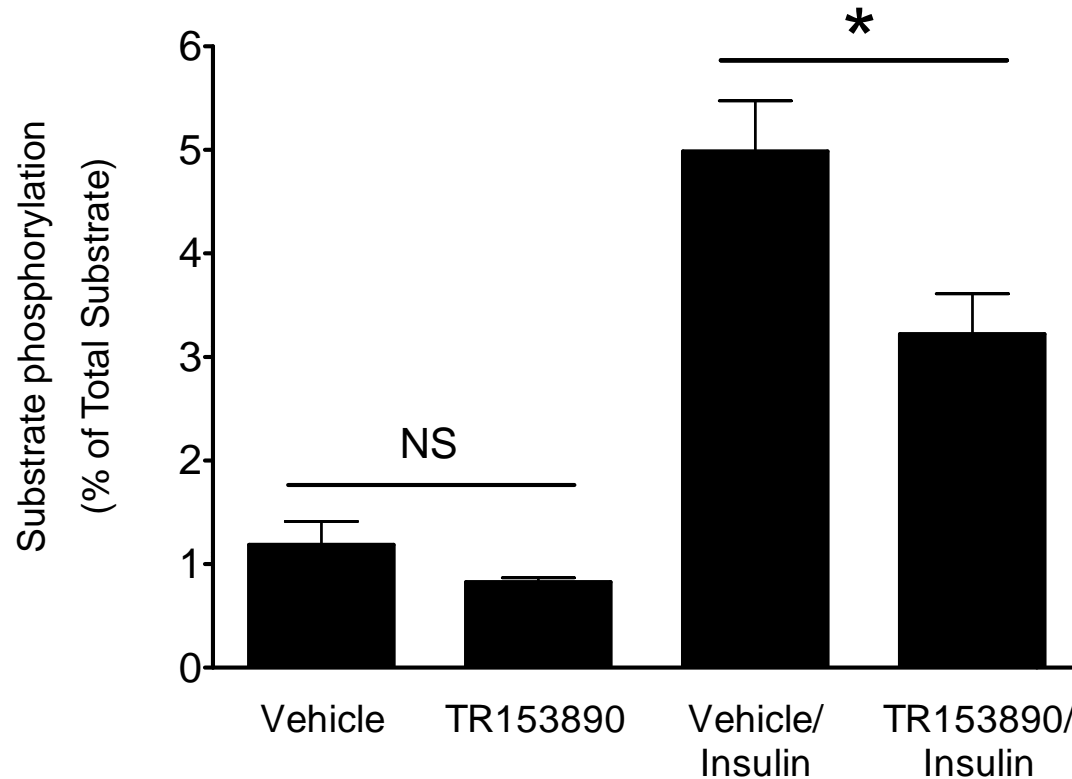
Cell-Based Profile Of TR153890 In CHO-IR Cells



■ **Statistically significant effect on:**

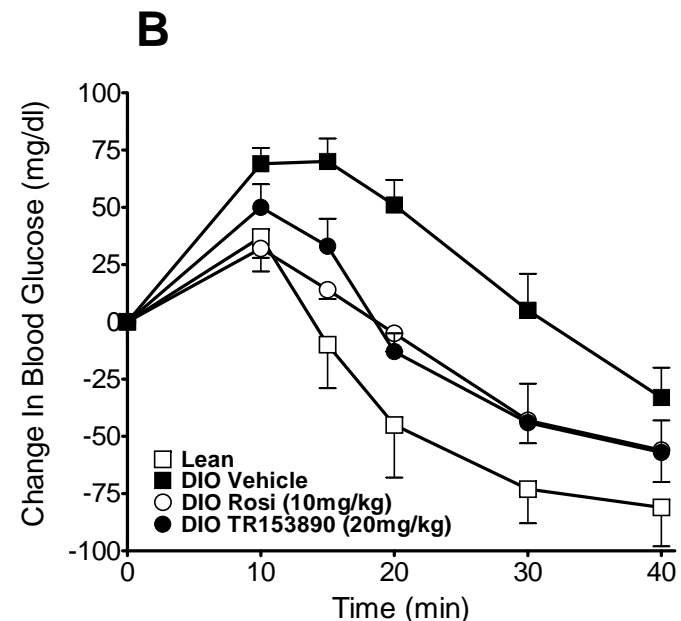
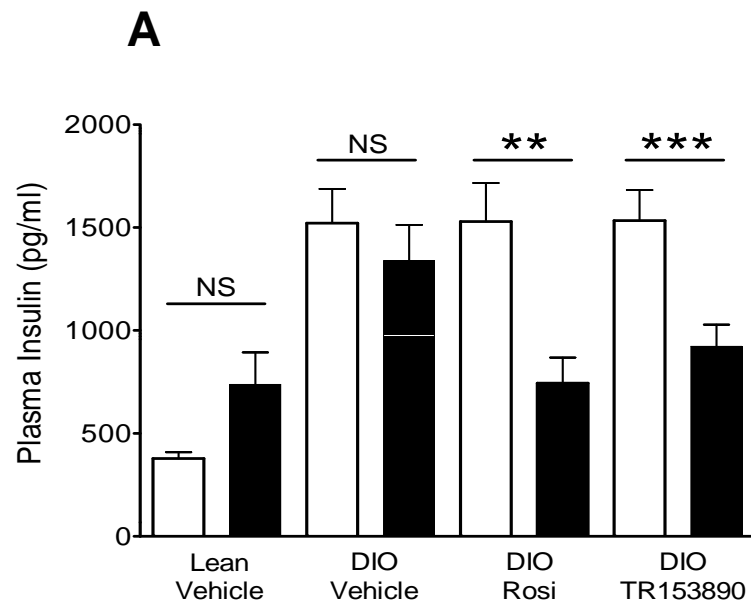
-	pSubstrate	$pIC_{50} = 7.23 \pm 0.12$	Inhibition	$= 144.9 \pm 2.7\%$	(n=4)
-	pIRS1 (302)	$pIC_{50} = 7.45 \pm 0.15$	Inhibition	$= 43.3 \pm 4.1\%$	(n=4)
-	pAkt	$pEC_{50} = 7.92 \pm 0.05$	Enhancement	$= 29.1 \pm 6.8\%$	(n=4)
-	pEnzyme	$pIC_{50} = 8.47 \pm 0.05$	Inhibition	$= 100 \%$	(n=4)

TRL153098 Reduced Insulin-Stimulated pSubstrate Levels in Quadriceps Muscle



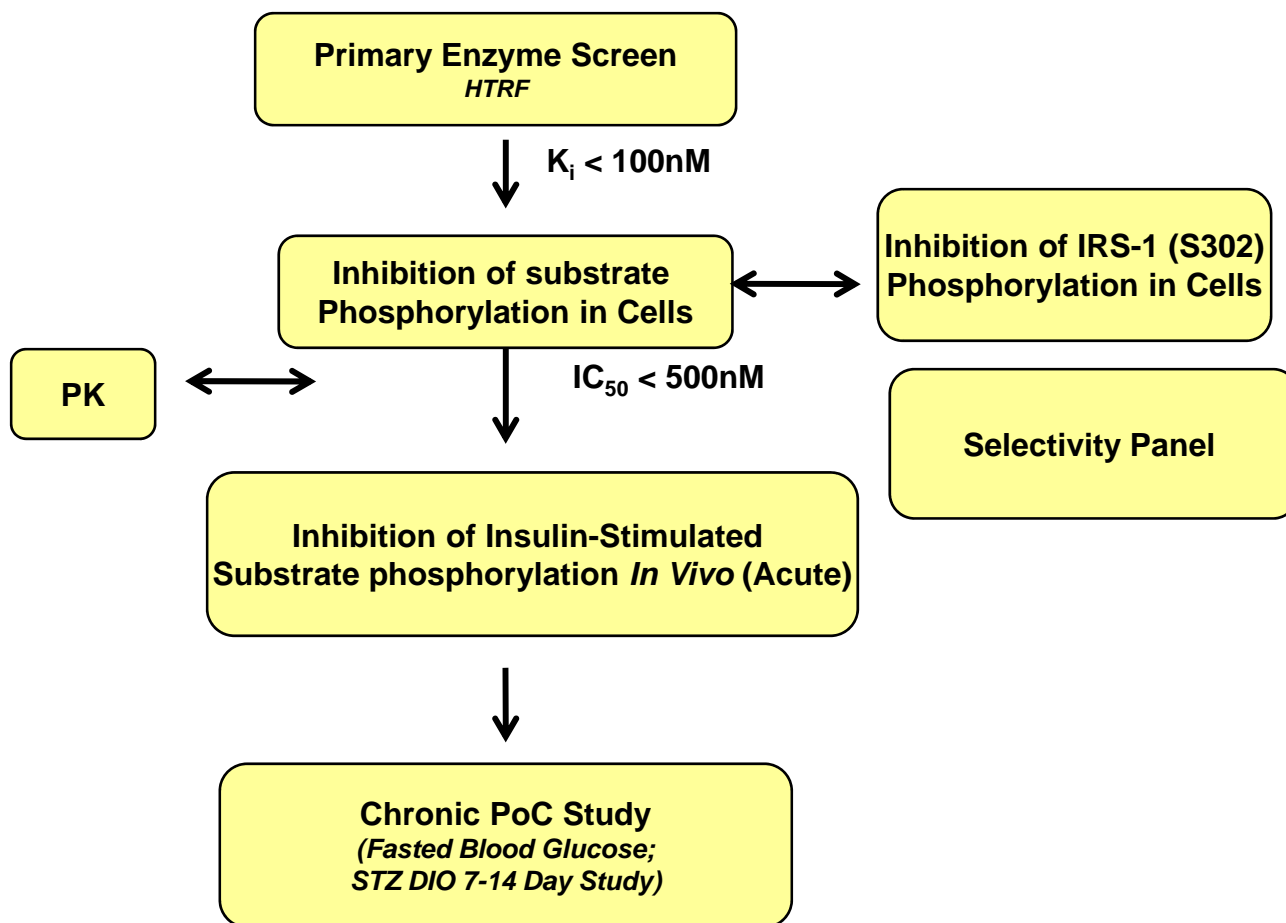
BALB/c mice treated acutely with TR153098 (20mg/kg; 30min)

Profile Of TR153098 In Preliminary DIO Mouse Study



- (A) In DIO C57BL6/J mice treated with TR153098 (20mg/kg; BID; 14 days) there was a significant decrease in fasting insulin levels
- (B) In same DIO mice, TR153098 produced a significant increase in insulin sensitivity in an *ip*ITT
- No effect of compound treatment on food intake or body weight over 14 day treatment

Compound Profiling - Pathway To TR153098



Compound X is Potent, Selective Inhibitor

-In Vitro Analysis

- Concentration-dependent decrease in kinase enzyme activity by ATP competitive mechanism
- Highly selective against panel of 120 kinases using Caliper Profiler Pro Plates
- Concentration-dependent decrease in insulin-stimulated phosphorylation of substrate in C2C12 mouse myoblasts
- Enhancement in pAKT levels in 3T3-L1 and HepG2 cells (25%)
- Decrease in pIRS (S302) in CHO-IR cells (43%)

TRL153098, Selective Inhibitor

-In Vivo Analysis

- Significant decrease in phosphorylation of ribosomal protein in quadricep muscle of mice treated with insulin
- Effectively lowered fasting glucose levels to lean control values In DIO model of insulin resistance after 14-day treatment
- No Significant change in body weight or food intake

Acknowledgements

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