New Insulin Sensitizers Produce Differentiation of Brown-like Adipose Cells from a Subcutaneous Fat Depot and Increase Secretion of Adiponectin *in vitro*

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47th EASD Annual Meeting
Lisbon, Portugal
September 12 - 16 2011

Presenter Disclosure

Jerry R. Colca, PhD

Board Member/Cofounder: **Metabolic Solutions Development Co., LLC**
Employee: **Metabolic Solutions Development Co., LLC**
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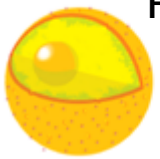
Mechanism of Action for Insulin Sensitizers

Old

Troglitazone; Rosiglitazone; Pioglitazone

Original TZDs

PPARγ



PPAR-Driven Gene Changes

Fat Sequestered
Increased Insulin Action
Fluid Retention
Weight Gain

New

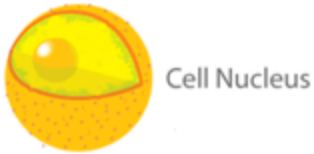
MSDC

Mito Target of TZDs (mTOT)

MSDC-0160
MSDC-0602

(Phase 2 clinical trials)

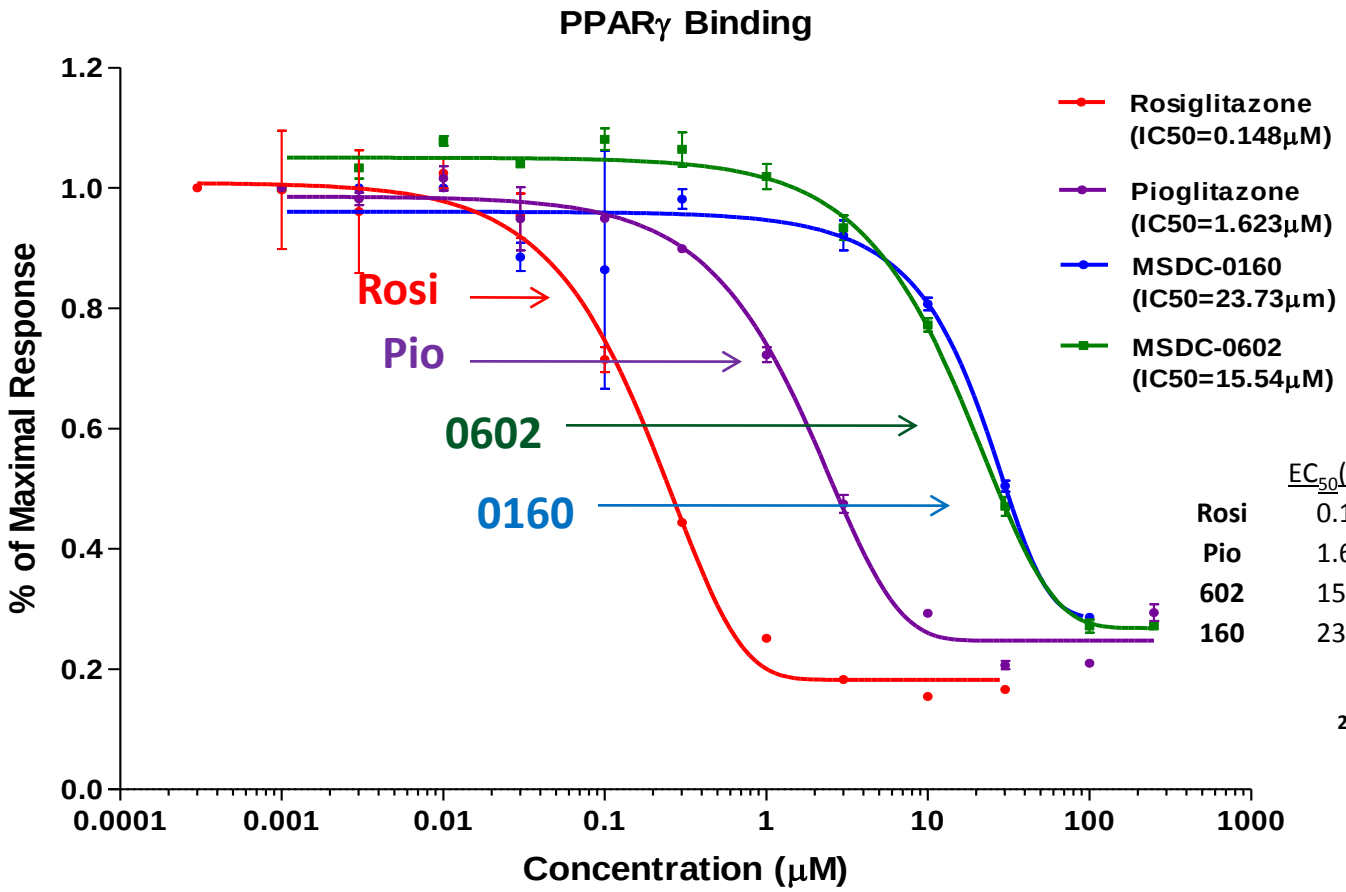
Metabolic signals



Nuclear Regulatory Factors

Improved Insulin Action
Improved Lipid Profiles
Regeneration of Brown Fat
Regeneration of β-cells

Relative Activity Against PPARγ



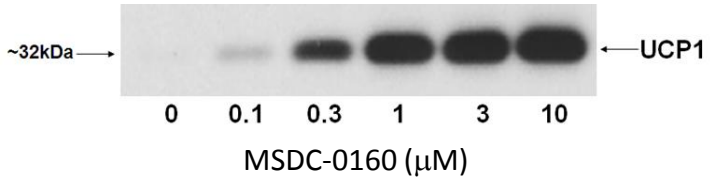
	Binding	Activation
	EC_{50}(μM)	Fold rosi[1]	Fold rosi[2]
Rosi	0.148	1.00	1.00
Pio	1.623	11	3
602	15.54	**105**	**39**
160	23.73	**160**	**29**

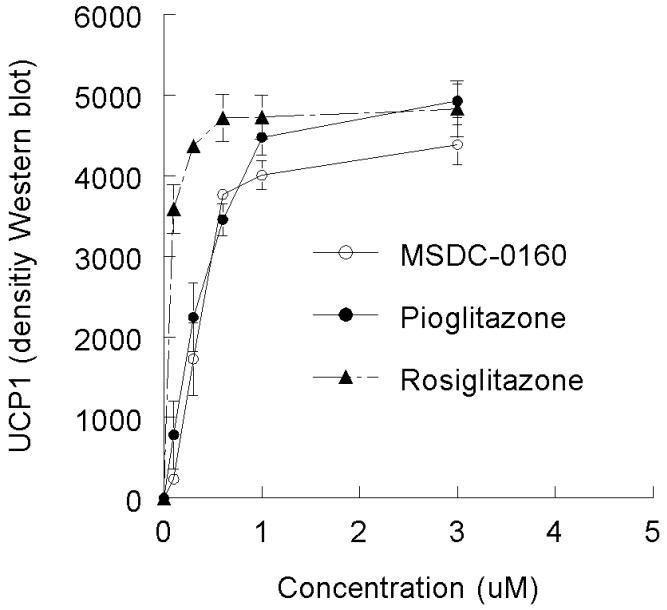
[1] Lantha screen –binding
[2] Gene blazer- cell activation

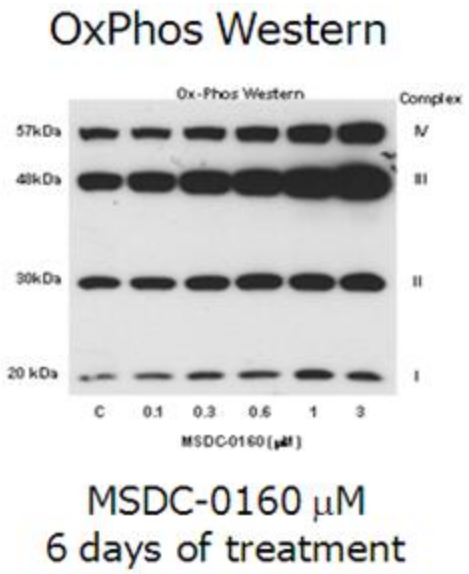
This Presentation:

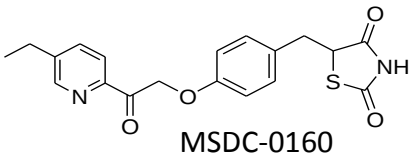
Compounds also stimulate brown-like phenotype in precursors from axillary fat pads and stimulate production and secretion of adiponectin in a PPARγ-independent manner.

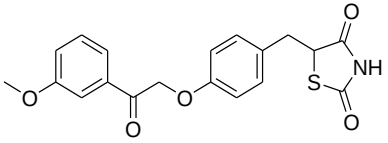
TZDs Increase Differentiation of Brown Fat Progenitors

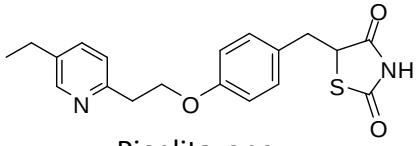


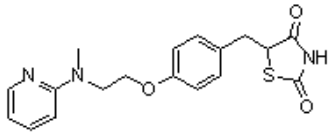










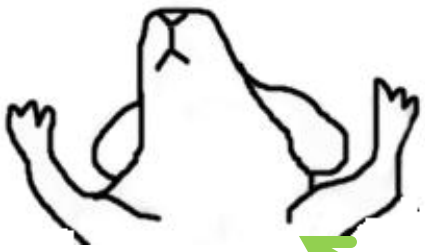


- The effect of TZDs on BAT is maintained in new insulin sensitizers.
- Similar to pio and rosi (although > 30-fold, 10-fold reduction at PPARγ vs rosi, pio).
- Not blocked by PPARγ antagonists; signaling occurs in PPARγ-KO cells.

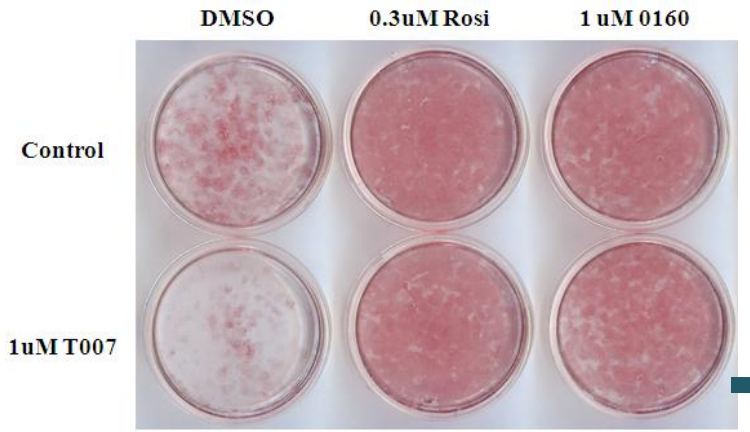
Will new insulin sensitizers affect subcutaneous fat ?
> Adiponectin production/secretion ?

Methods

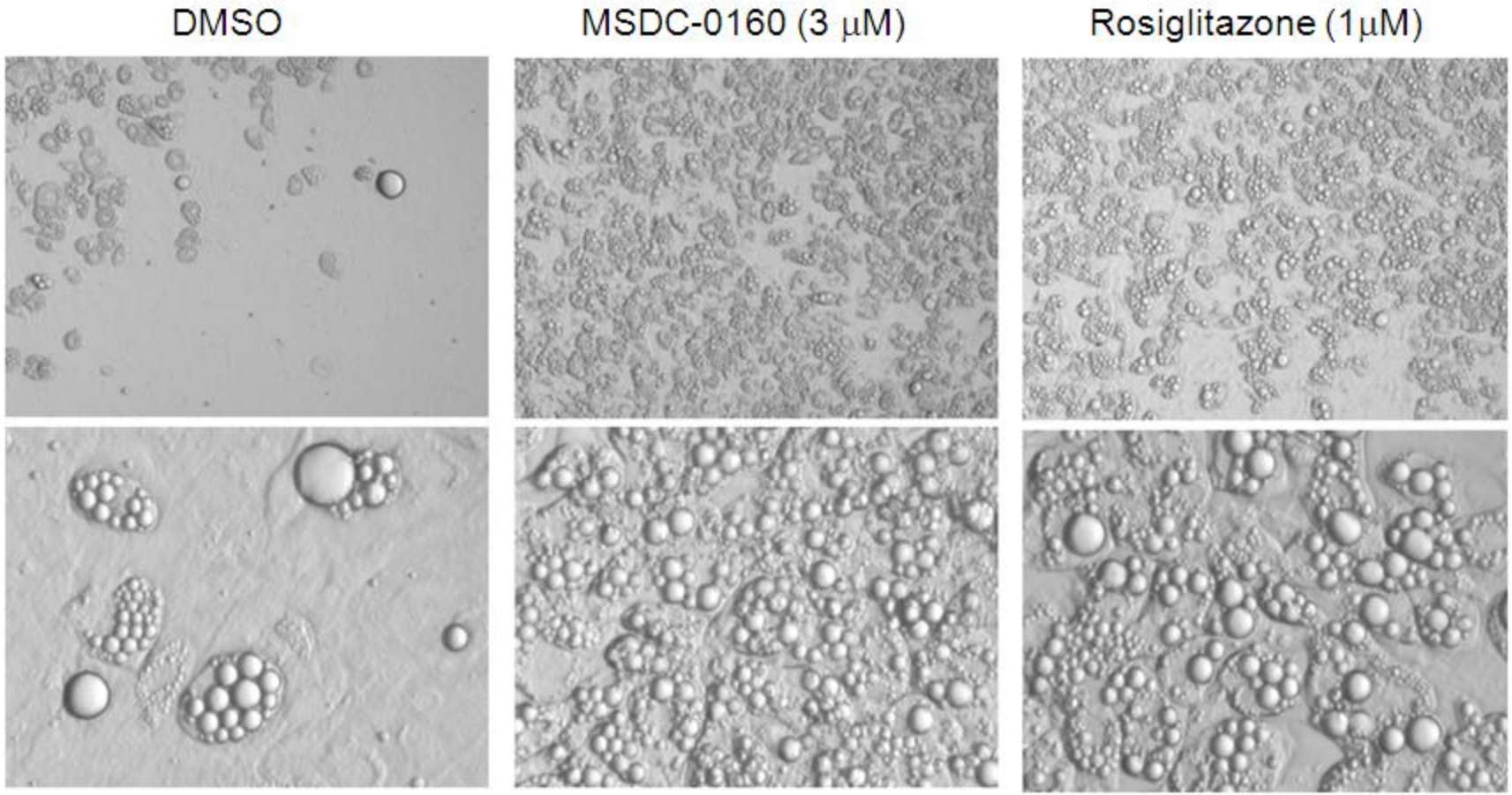
- **Progenitor cells are isolated from axillary fat pads from 3-4 week old CD-1 mice and cultured for 7 days in DMEM + 10% FBS.**
- **At 90% confluence the cells are treated with various concentrations of compounds (172 nM insulin); medium is changed every 48 hours with fresh additions.**
- **Cells are harvested for mRNA analysis (rt-PCR) and Western Blots at various time points.**
- **Conditioned medium is harvested for measurement of secreted adiponectin by ELISA .**



- Pad isolated from 10-15 mice
- Digested with collagenase
- Precursors isolated and plated
- Grown to 90% confluence



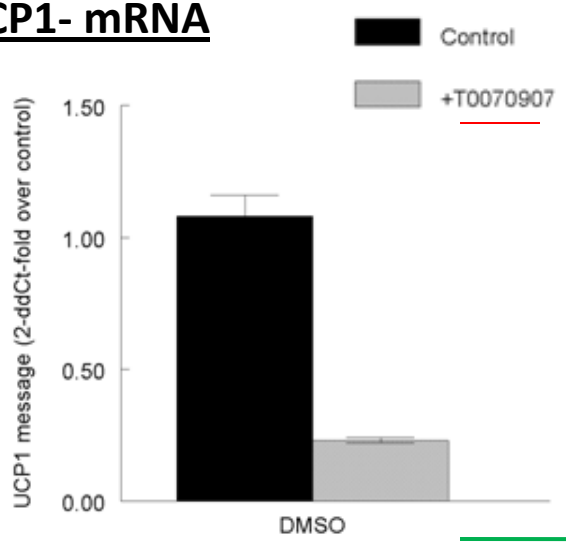
Conversion of Progenitor Cells to Brown-like Phenotype (7 days of treatment)

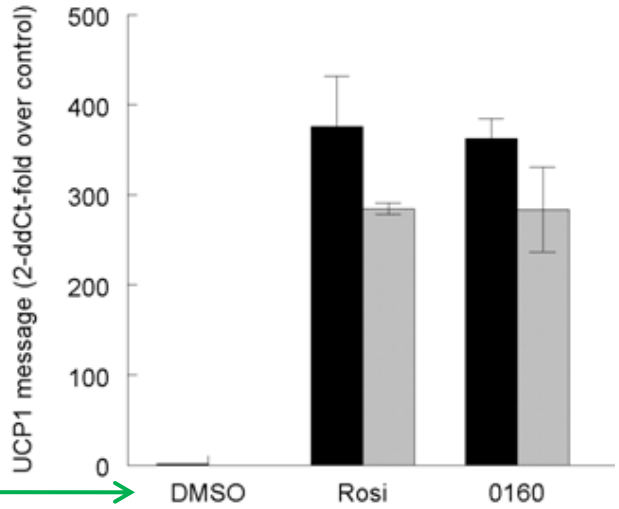


- Multilocular fat droplets
- Increased Mitochondria
- Increased UCP1 (message and protein)
- Increased adiponectin (message, protein, and secretion)

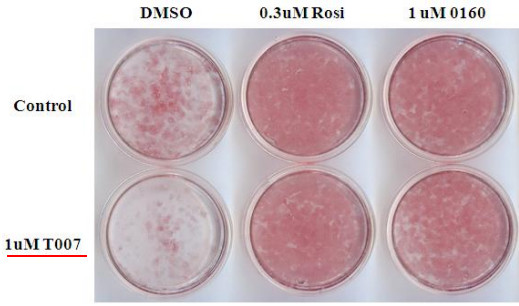
PPARγ Antagonists Do Not Block Compound-Induced Effects on UCP1

UCP1- mRNA

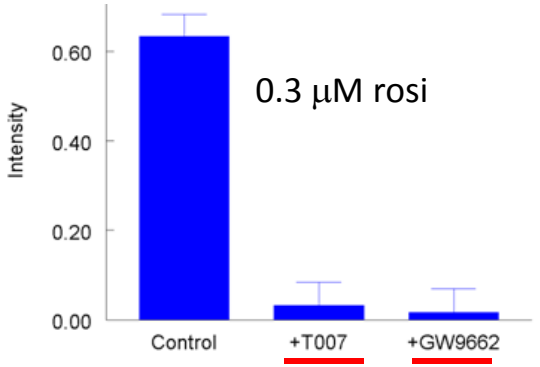




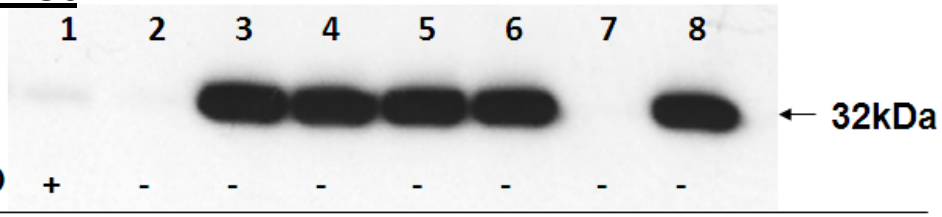
Note: antagonists affect baseline UCP1 mRNA and differentiation in absence of compounds



and rosi action in a PPARγ cell assay



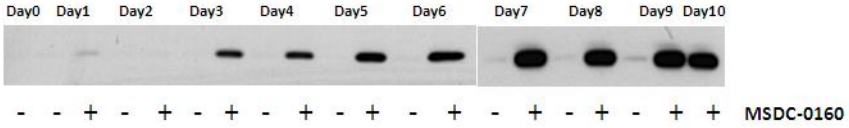
UCP1- Western Blot



DMSO	+	-	-	-	-	-	-	-
T0070907 (1μM)	-	+	-	-	+	+	-	-
Rosi (0.3 μM)	-	-	+	-	+	-	-	+
MSDC-0160 (1 μM)	-	-	-	+	-	+	-	-
GW9662 (1μM)	-	-	-	-	-	-	+	+

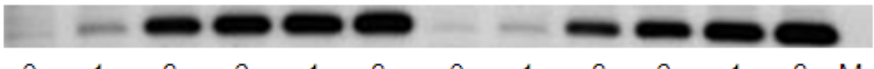
but do *not* block the effects of the compounds to increase UCP1 mRNA and protein.

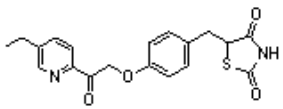
Increase in UCP1 Protein Expression in Subcutaneous Adipose Progenitors



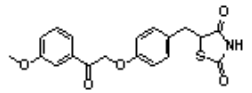
Time (days)

Western blot UCP1- 6 days

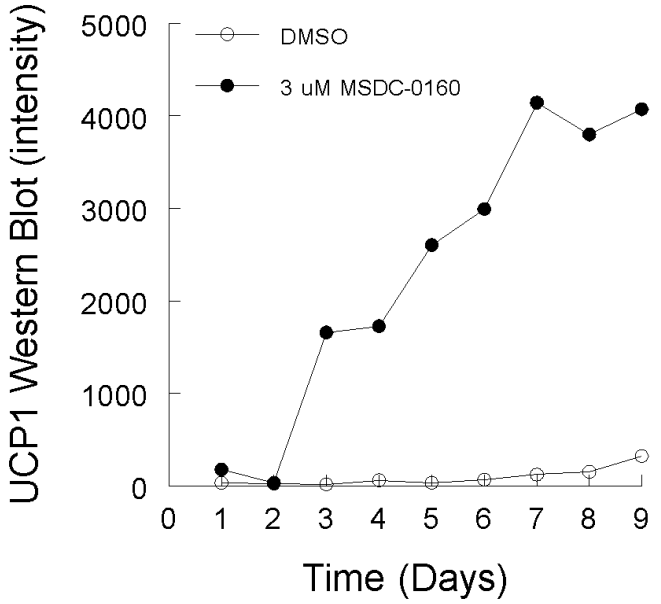




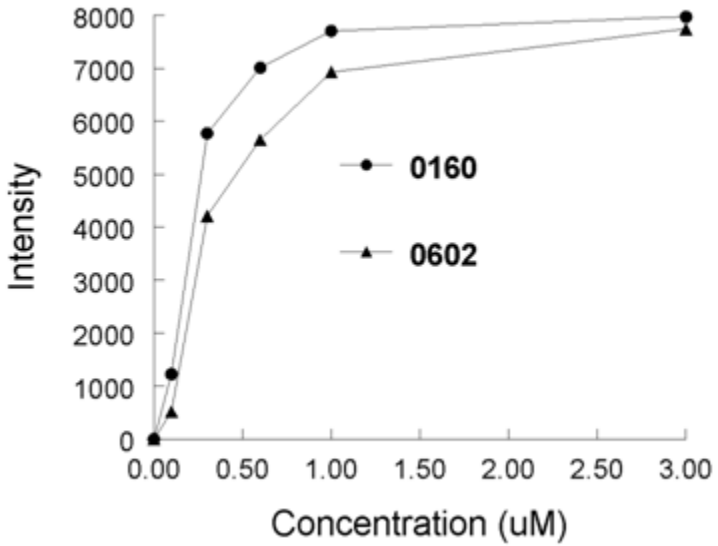
MSDC-0160



MSDC-0602



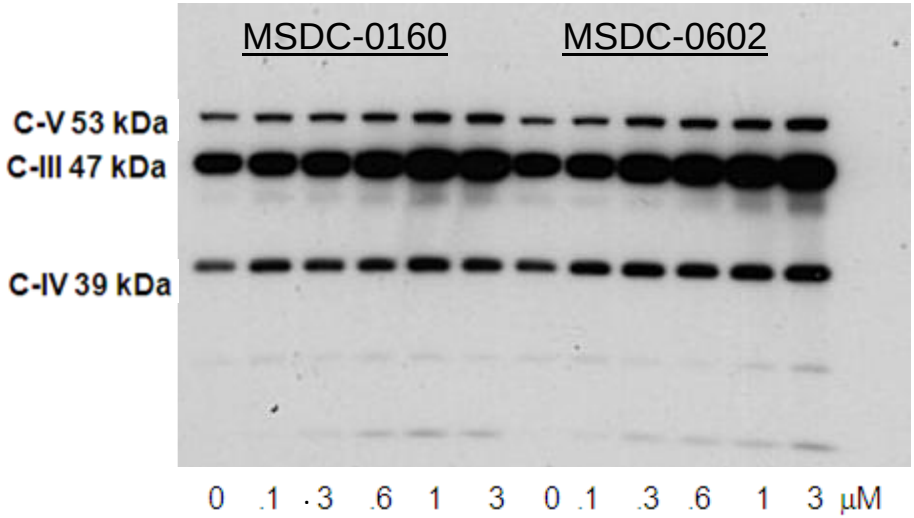
Dose (µM)



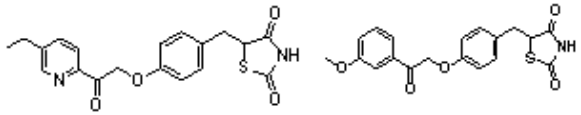
New Insulin Sensitizers Increase UCP1 Message and Protein In SC Adipose

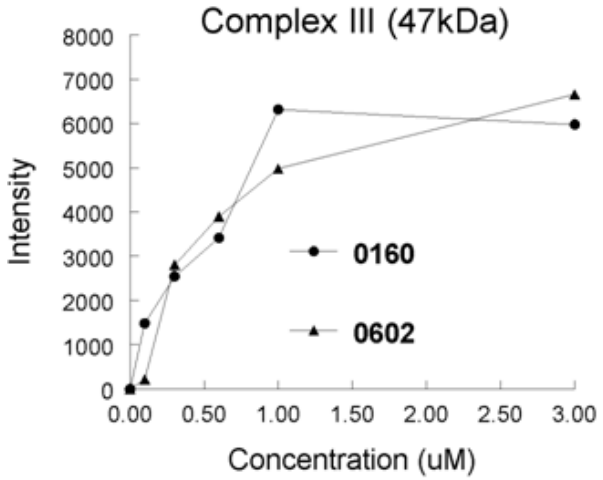
Increased Mitochondria in Subcutaneous Adipose Progenitors

CD-1 mice

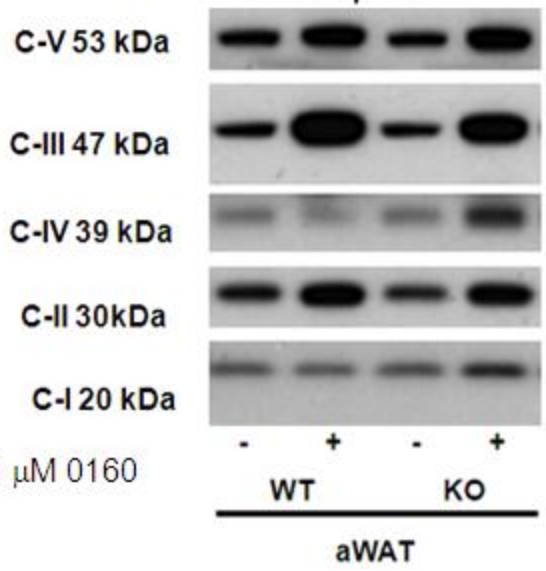


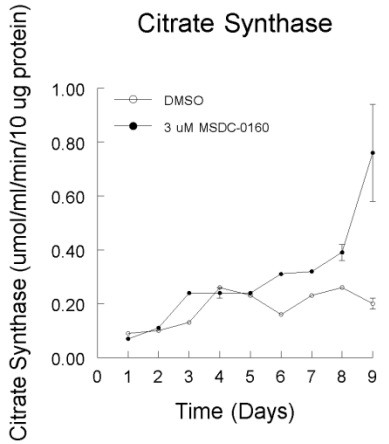
MSDC-0160





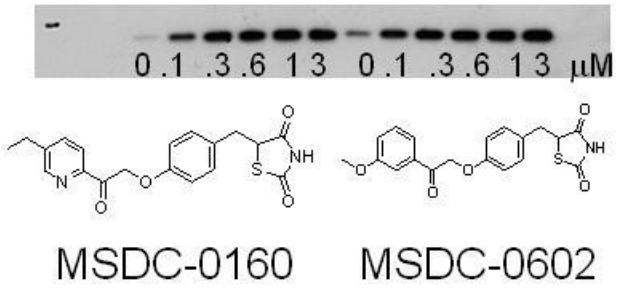
C57 mice-
PPARγ KO

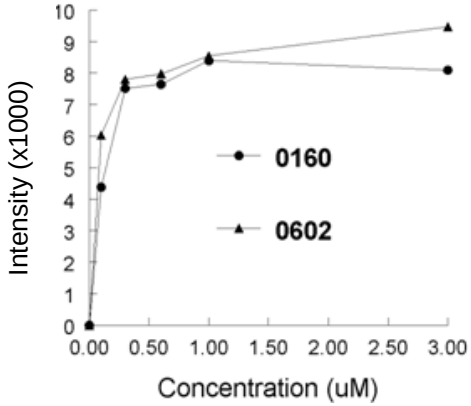




New Insulin sensitizers also increase mitochondrial biogenesis by a mechanism independent of PPARγ activation in SC adipose progenitors.

Increased Adiponectin Production and Secretion

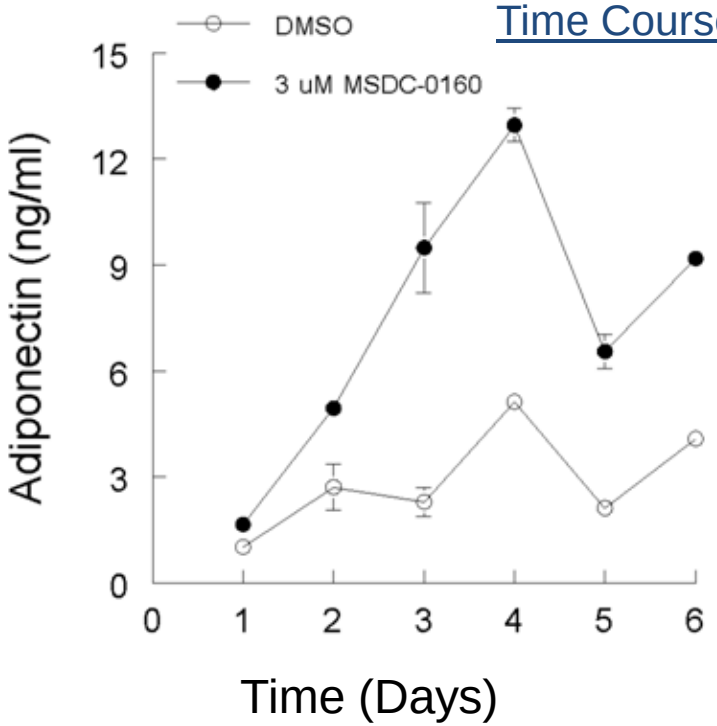




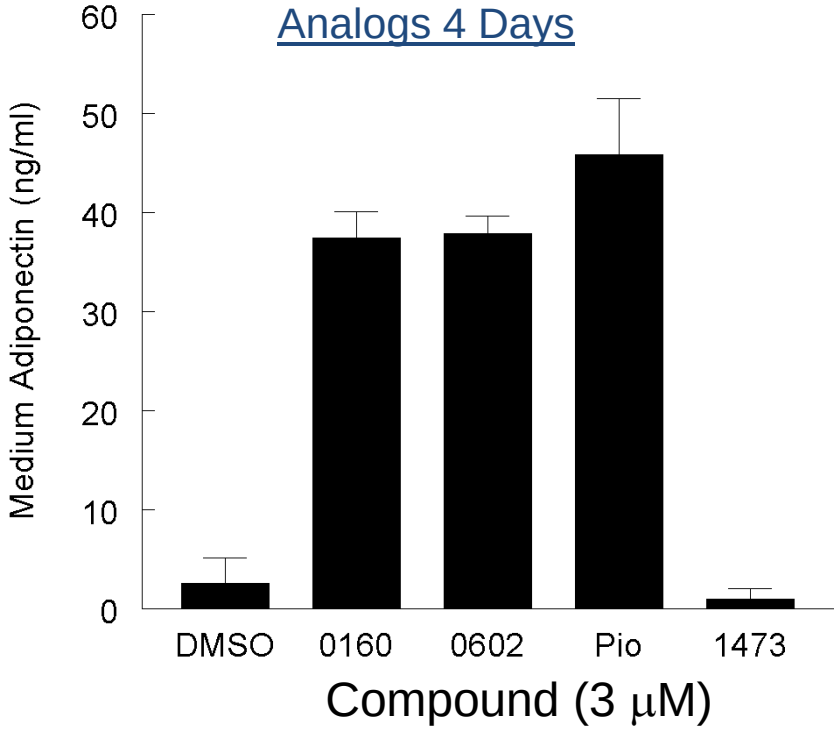
Western blot

Secretion

Time Course



Analogs 4 Days



New insulin sensitizers directly increase adiponectin production in subcutaneous adipose Independent of expansion of white fat or activation of PPARγ.

Summary

- **New insulin sensitizing agents cause browning of progenitor cells from the axillary fat pad in a PPAR-independent manner.**
  - Not related to ability to bind to and activate PPARγ - rosi vs 0160 and 0602
  - Not blocked by PPARγ antagonists
- **This mechanism includes increase in UCP1 and mitochondrial biogenesis.**
  - mRNA
  - Protein
- **The compounds increase adiponection in a PPARγ-independent manner.**
  - Expression
  - Secretion into the medium

❖ New insulin sensitizers not only stimulate differentiation of dedicated brown fat progenitor cells but also favor brown adipose-like phenotype and increase adiponectin secretion from subcutaneous adipose.

❖ There is potential for a new generation of insulin sensitizing agents that avoids side effects associated with activation of PPARγ.

Implications for Novel Agents