



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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Presenter Disclosure

Jerry R. Colca, PhD

Board Member/Cofounder: **Metabolic Solutions Development Co., LLC**

Employee: **Metabolic Solutions Development Co., LLC**

Stock/Shareholder: **Metabolic Solutions Development Co., LLC**

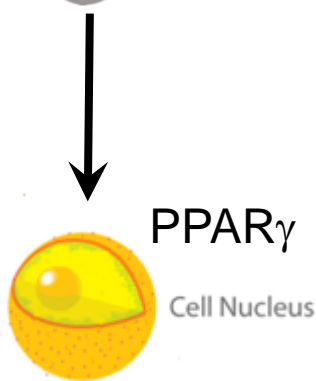


Mechanism of Action for Insulin Sensitizers

Old

Troglitazone; Rosiglitazone; Pioglitazone

 **Original TZDs**



PPAR-Driven Gene Changes

Fat Sequestered
Increased Insulin Action
Fluid Retention
Weight Gain

New

 **MSDC**

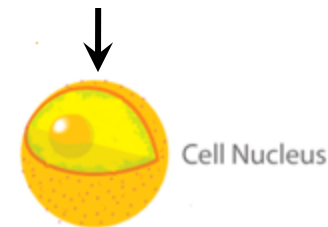
Mito Target of TZDs (mTOT)



MSDC-0160
MSDC-0602

(Phase 2 clinical trials)

Metabolic signals

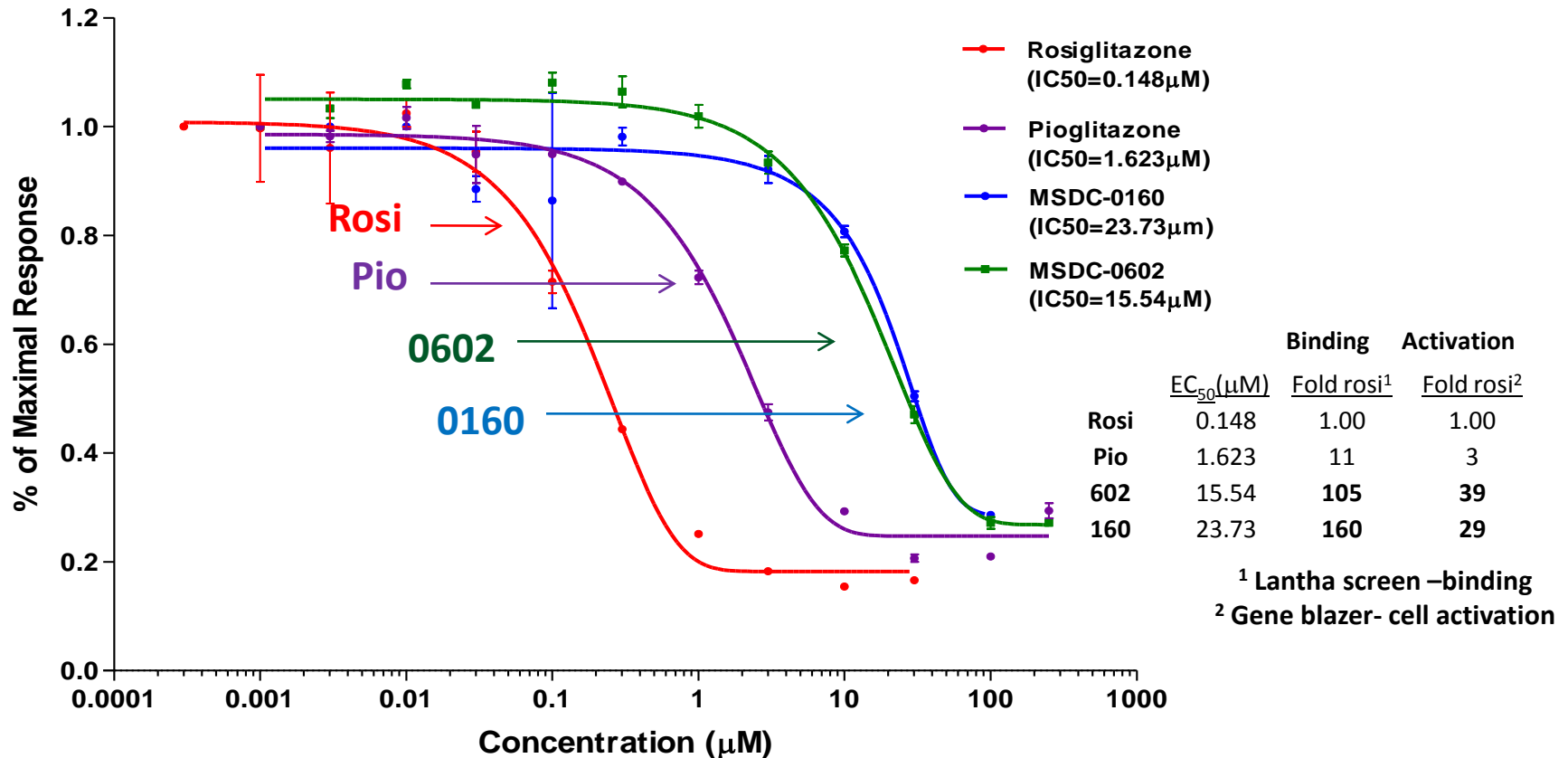


Nuclear Regulatory Factors

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

Relative Activity Against PPAR γ

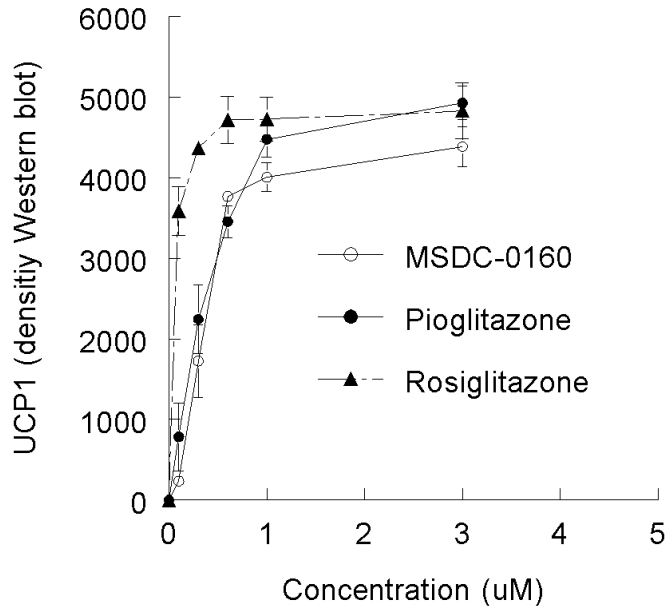
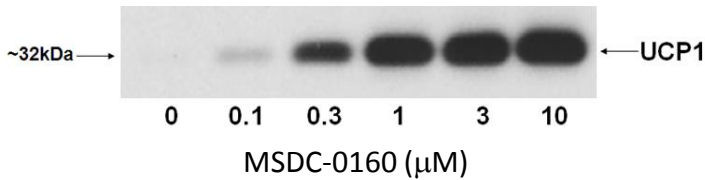
PPAR γ Binding



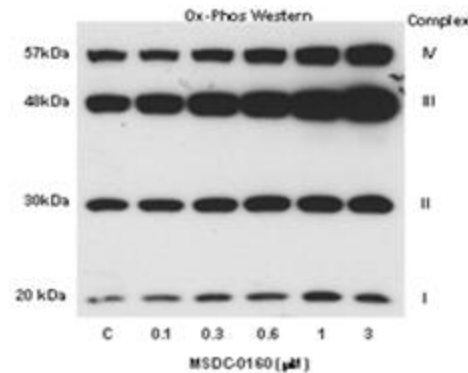
This Presentation:

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

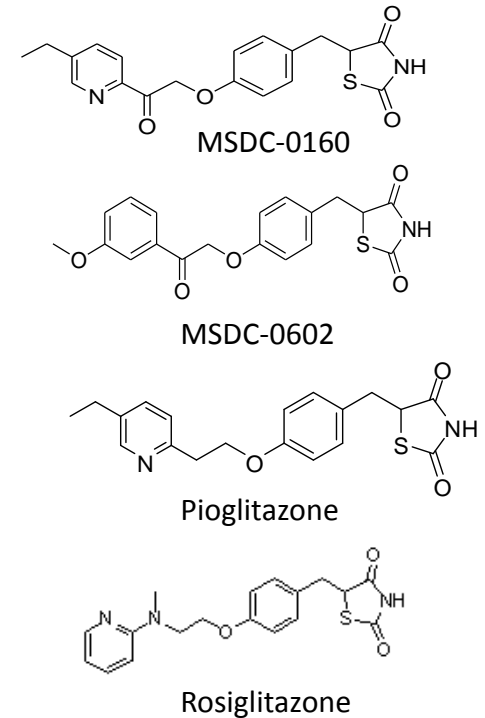
TZDs Increase Differentiation of Brown Fat Progenitors



OxPhos Western



MSDC-0160 μM
6 days of treatment

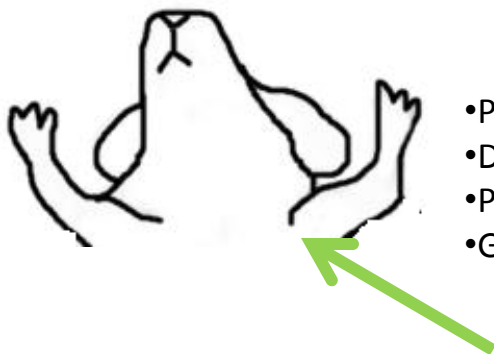


- The effect of TZDs on BAT is maintained in new insulin sensitizers.
- Similar to pio and rosi (although > 30-fold, 10-fold reduction at PPAR γ vs rosi, pio).
- Not blocked by PPAR γ antagonists; signaling occurs in PPAR γ -KO cells.

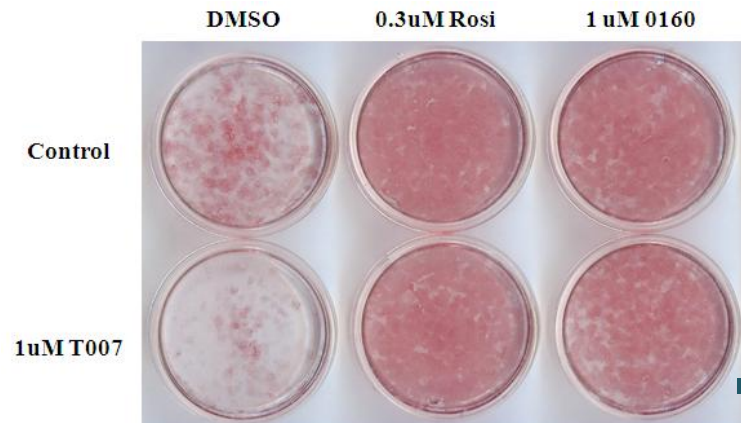
Will new insulin sensitizers affect subcutaneous fat ?
> Adiponectin production/secretion ?

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



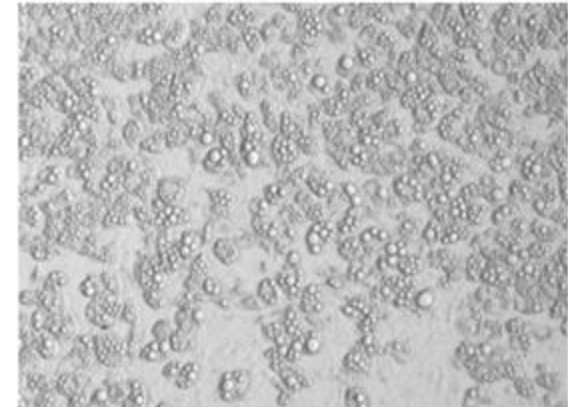
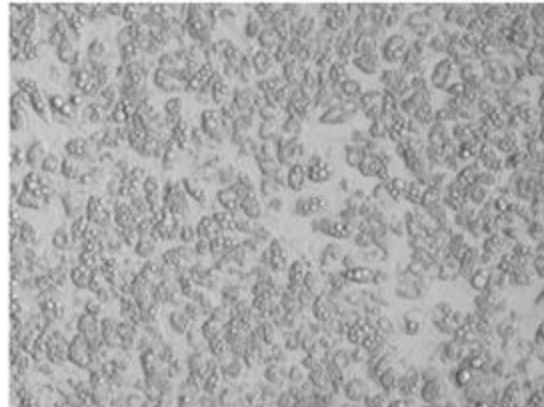
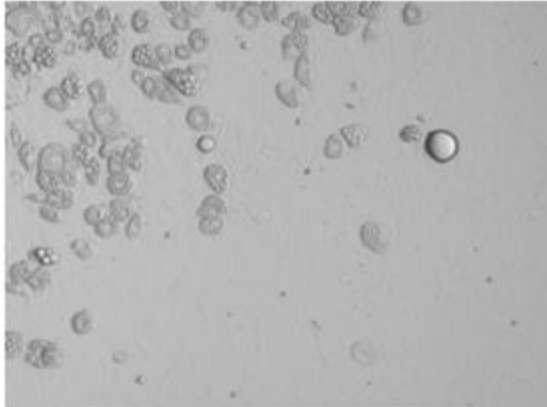
Conversion of Progenitor Cells to Brown-like Phenotype (7 days of treatment)

DMSO

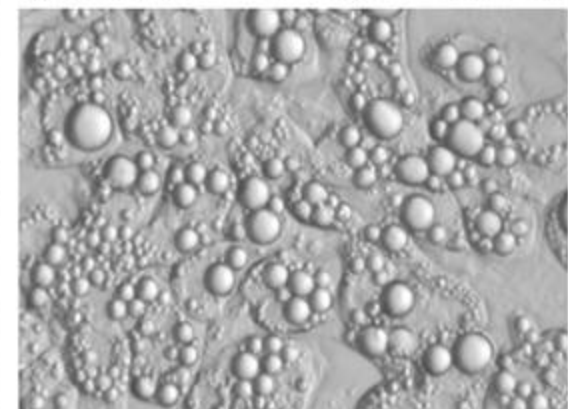
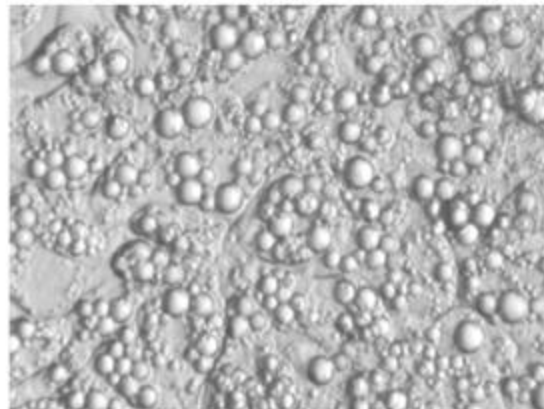
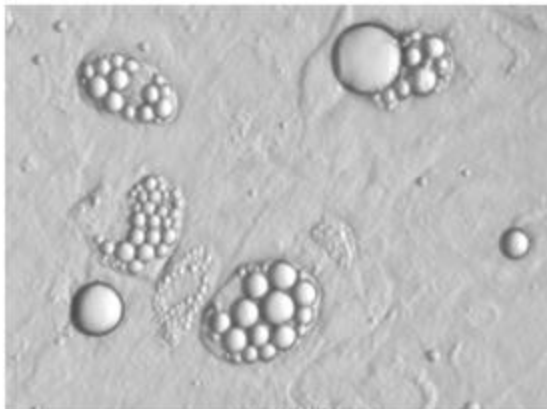
MSDC-0160 (3 μ M)

Rosiglitazone (1 μ M)

10x



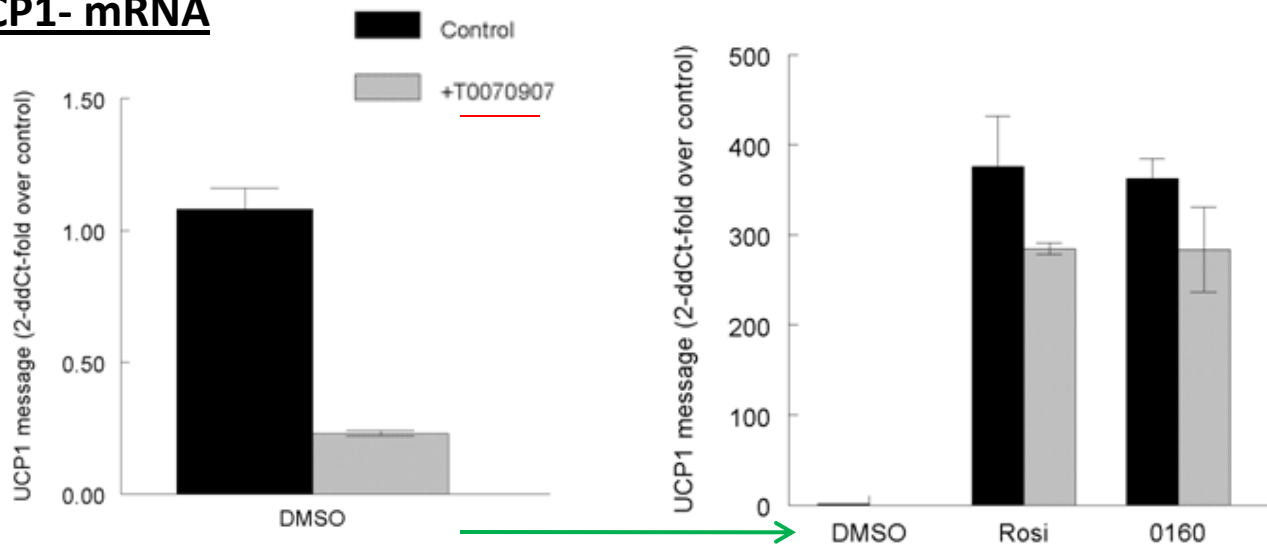
40x



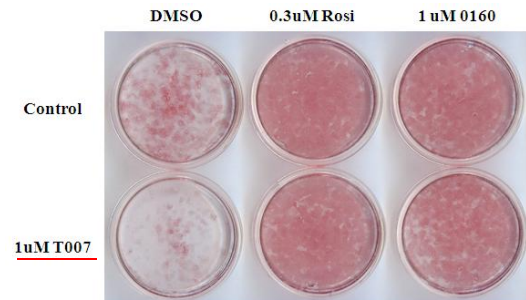
- Multilocular fat droplets
- Increased Mitochondria
- Increased UCP1 (message and protein)
- Increased adiponectin (message, protein, and secretion)

PPAR γ Antagonists Do Not Block Compound-Induced Effects on UCP1

UCP1- mRNA

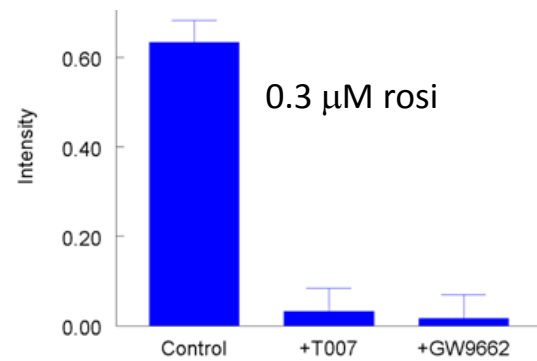
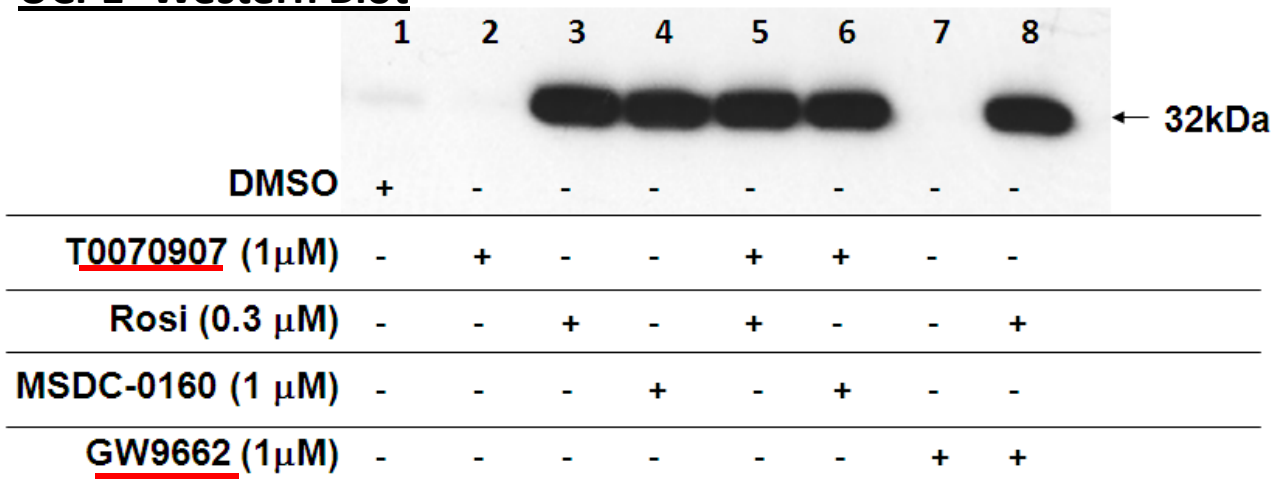


Note: antagonists affect baseline UCP1 mRNA and differentiation in absence of compounds



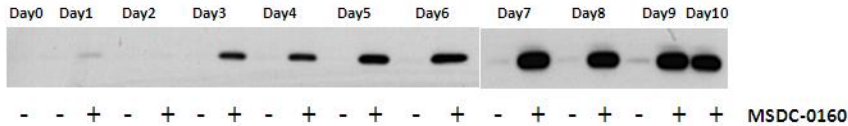
and rosi action in a PPAR γ cell assay

UCP1- Western Blot

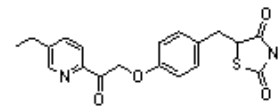
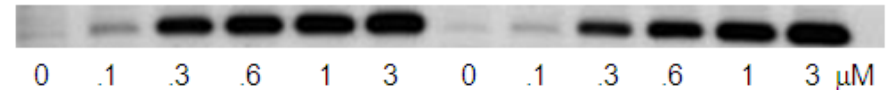


but do *not* block the effects of the compounds to increase UCP1 mRNA and protein.

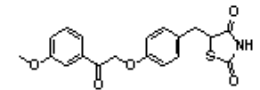
Increase in UCP1 Protein Expression in Subcutaneous Adipose Progenitors



Western blot UCP1- 6 days

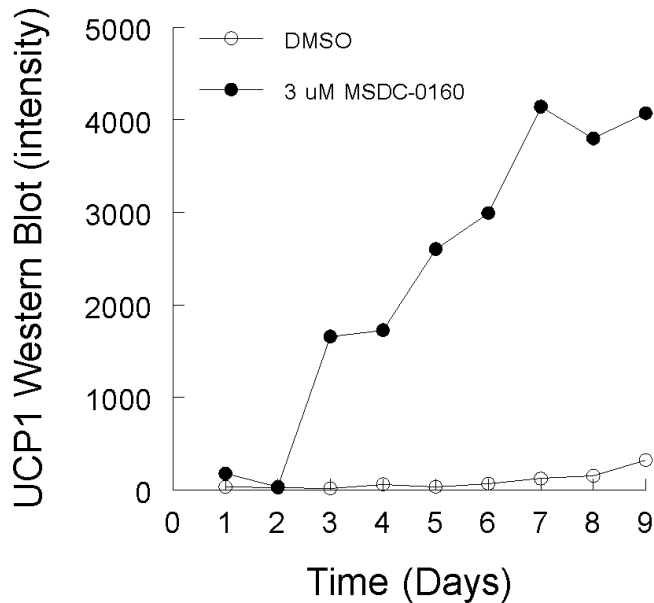


MSDC-0160

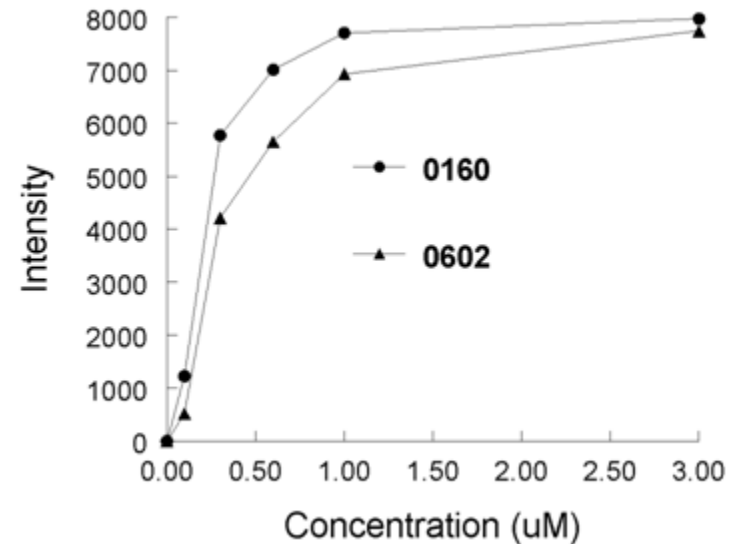


MSDC-0602

Time (days)



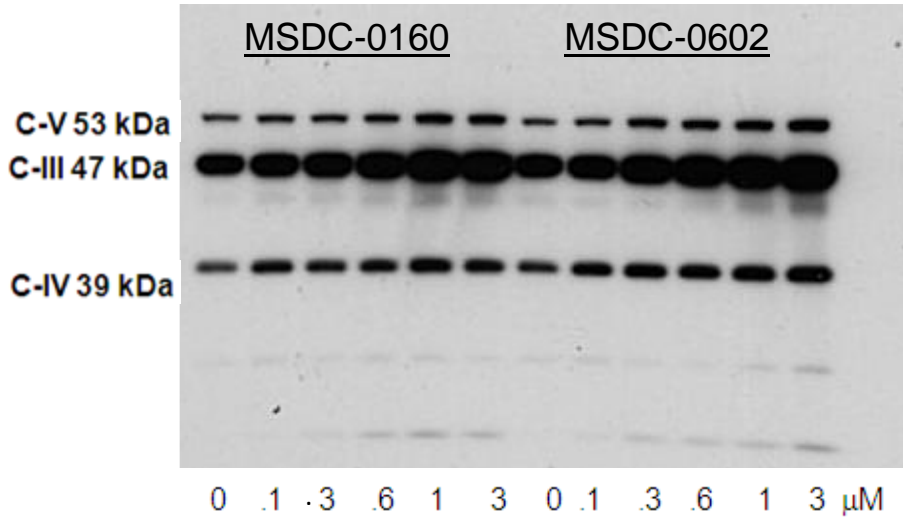
Dose (μM)



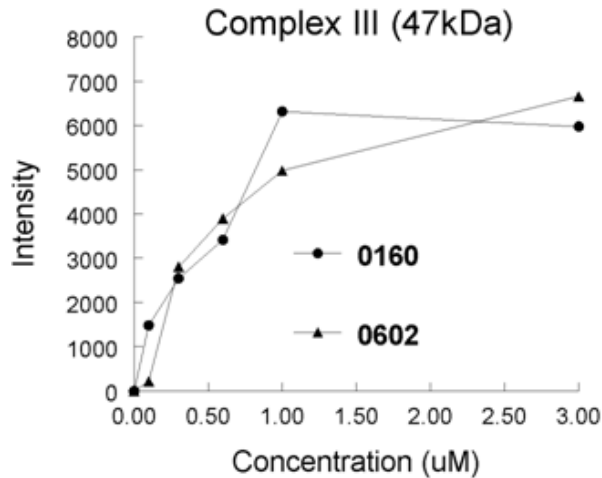
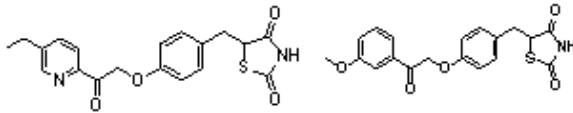
New Insulin Sensitizers Increase UCP1 Message and Protein In SC Adipose

Increased Mitochondria in Subcutaneous Adipose Progenitors

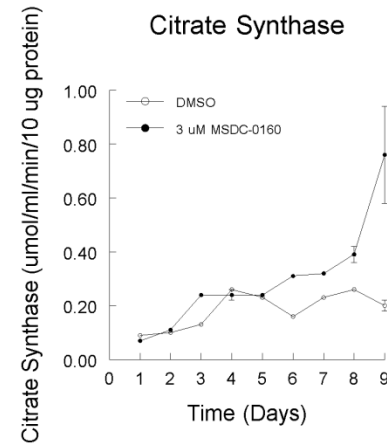
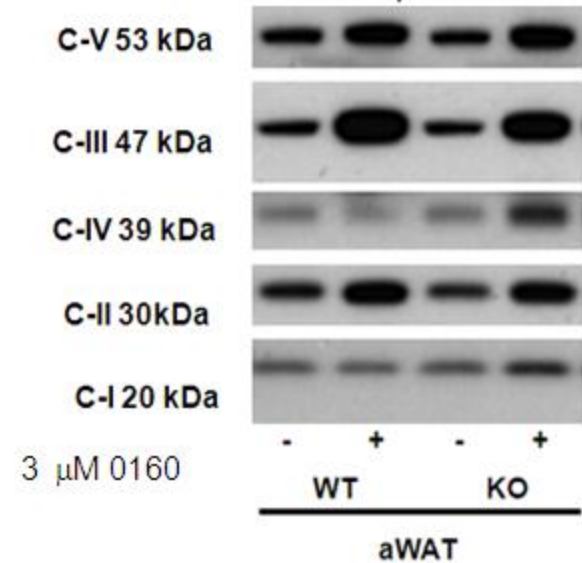
CD-1 mice



MSDC-0160

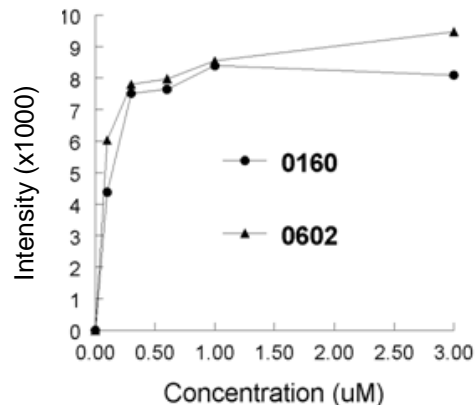
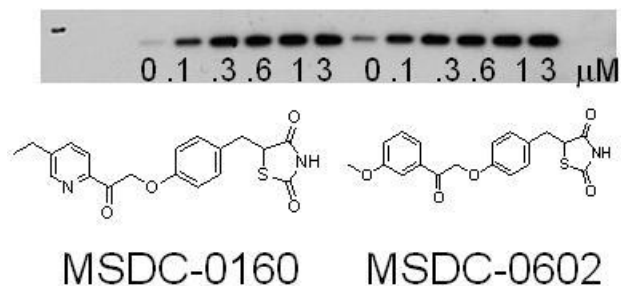


C57 mice-
PPAR γ KO



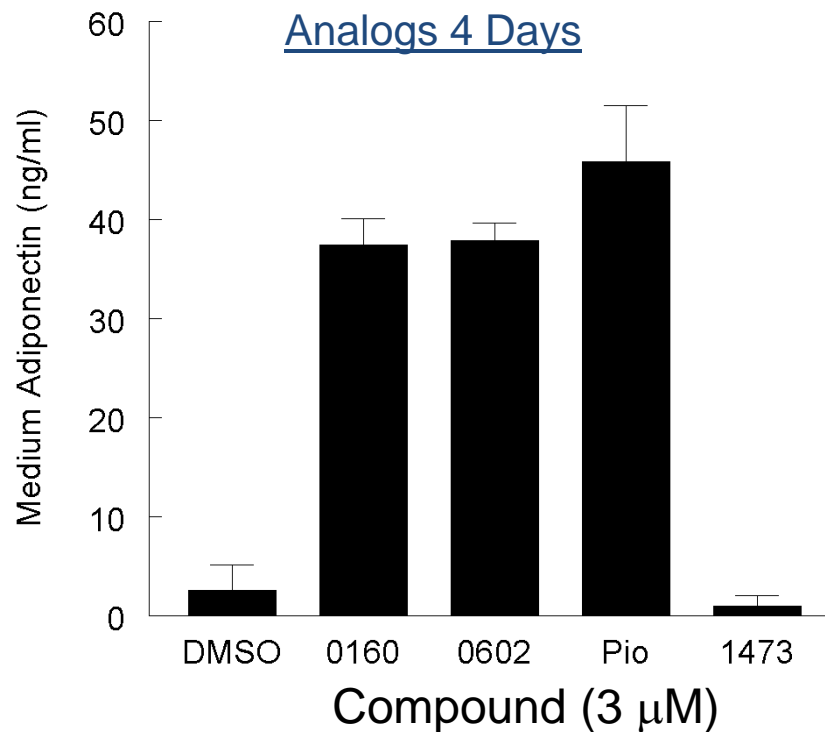
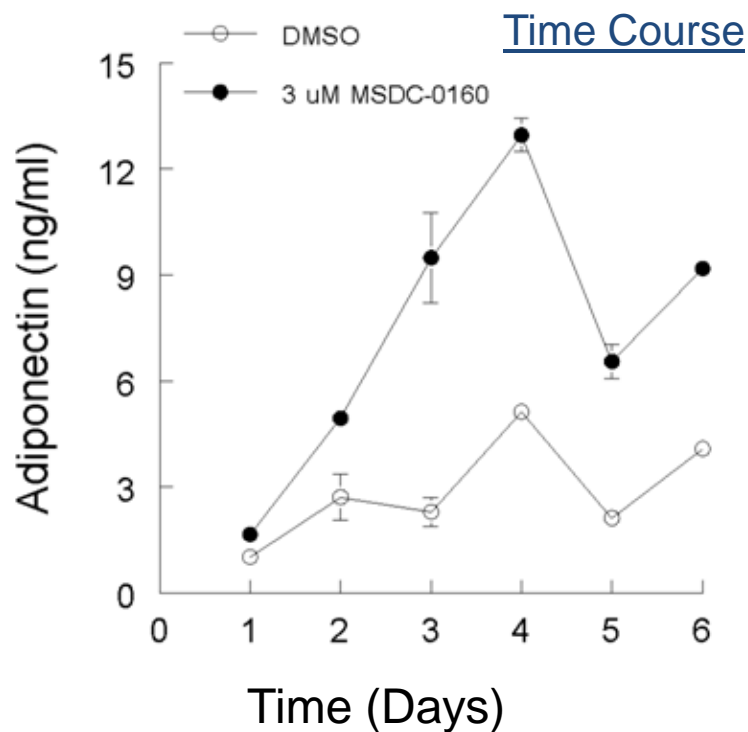
New Insulin sensitizers also increase mitochondrial biogenesis by a mechanism independent of PPAR γ activation in SC adipose progenitors.

Increased Adiponectin Production and Secretion



Western blot

Secretion



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

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 PPAR γ - rosi vs 0160 and 0602
 - Not blocked by PPAR γ antagonists
- **This mechanism includes increase in UCP1 and mitochondrial biogenesis.**
 - mRNA
 - Protein
- **The compounds increase adiponectin in a PPAR γ -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 PPAR γ .

Implications for Novel Agents

