Novel Insulin Sensitizers

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Presentation

- PPARG Modulators devoid of classical Agonism
 - Insulin sensitizers and nuclear receptors
 - PTM control of NR Signaling and SR1664
 - Structural aspects of SR1664 Action
 - Potential to develop novel non-agonists PPARG modulators for use in the clinic?

Discovery of Insulin Sensitizing TZDs

Thiazolidinediones (TZDs)





Ikeda et al. Diabetes 1981



Classical Model of NR Signaling



Proposed Mechanism of Action of TZDs



PPARG:RXR Crystal Structure





PPARG Ligands and Insulin Resistance

Partial loss of function mutations in PPAR γ in humans unambiguously cause severe insulin resistance. But....

PPAR γ agonists improve insulin-resistance and diabetes, but most PPAR γ target genes are already fully "on" in obesity – and typically there is no defect in receptor.

Some PPARγ ligands with poor agonist activity still have *marked* anti-diabetic actions (MRL24, Mbx-102, INT131).

The PPARG Paradox



Paradox: If agonism of PPARG drives adipokine expression then why are partial agonists equally efficacious as full agonists?

Choi et al. Nature 2010

Questions about Partial Agonists

- Do PAs regulate expression of adipokines via a different mechanism than TZDs?
- Do PAs afford separation of insulin sensitization pathways from pro-adipogenic, fluid expansion/retention, cardiohypertrophy?



PTM status impacts PPARG function



Ligands bind to PPARG and interfere with the ability of kinase(s) to PO3 the receptor



Choi et al Nature 2010

PTM status impacts PPARG function



Modulation of PPARG phosphorylation by rosiglitazone during therapy of human type 2 diabetics



w/Matthias Blueher, U Leipzig

Choi et al, Nature 2010

PTM Control of NR Signaling



Combinatorial Control of NR Function

Functional Interactions

Ligand – AF2 Ligand – dimer partner AF1- AF2 Ligand – AF1 **Ligand – PTM**

PTM – co-regulator

DNA – co-regulator PTM – DNA Ligand – DNA Ligand-mediated translocation

Goal of our lab – to develop functionally selective modulators of nuclear receptors

PPARG LBD PTMs



S273: phosphorylation correlates with obese gene expression. *Choi et al. Nature 2010*

K365: SUMOylation leads to repression of NFkB target genes. *Pascual et al. Nature 2005*

<u>K293:</u> 'Ser273 phosphorylation correlates with Lys293 acetylation' *Qiang et al. Cell 2012*

<u>K268 & K293</u>: 'Deacetylation of PPPARG on Lys293 is required to recruit coactivator Prdm16, while deacetylation on Lys268 and Lys293 is required to clear corepressor NCoR.' *Qiang et al. Cell 2012*

Paradigm Shift? Modulate PTMs and not Receptor Activation



Can Agonism and Blocking S273-P be separated?



Ligand Discovery



Antidiabetic actions of a non-agonist PPARγ ligand blocking Cdk5-mediated phosphorylation

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SR1664 effect on Adipocytes



SR1664 and Analogs on Bone



SR1664 and Analogs on Bone



| − Fr0 − Fr0d chan − Cr0 − Cr0 | 00 | | 4 |
|--|----|---------|---|
| red S staining ge over DMSO) - 8.0 - 9.0 - 9 - 9.0 - 9.0 - 9.0 - - | T | ** T | т |

| | | ALP activity | | |
|-----------------|------------|--------------|---------|-------------|
| | | uU/min | р | Fold change |
| | DMSO | 3.80 | | |
| Full Agonist | RSG/10uM | 0.54 | 1.0E-07 | 0.141 |
| | RSG/1uM | 0.69 | 2.4E-07 | 0.182 |
| Partial Agonist | 1824/10uM | 5.08 | 0.01 | 1.337 |
| | 1824/1uM | 4.78 | 0.02 | 1.258 |
| Full Agonist | 2227/10uM | 1.16 | 0.00 | 0.305 |
| | 2227/1uM | 2.25 | 0.05 | 0.593 |
| Partial Agonist | MRL24/10uM | 2.19 | 0.08 | 0.576 |
| | MRL24/1uM | 2.02 | 0.01 | 0.533 |
| Non-Agonist | 1664/10uM | 4.66 | 0.56 | 1.226 |
| | 1664/1uM | 4.54 | 0.32 | 1.194 |
| Non-Agonist | 2539/10uM | 4.14 | 0.71 | 1.089 |
| | 2539/1uM | 3.54 | 0.78 | 0.933 |
| | | | | |

Beata Lecka-Czernik in U-33/PPARg2 cells

Adipogenesis only seen with rosi

activity of pro-osteoblastic signaling - repression%

| Rosi | 50% |
|--------|-----|
| SR1824 | 30% |
| SR2227 | 30% |
| MRL24 | 20% |
| SR1664 | ~2% |
| SR2539 | 0% |
| | |

Inhibition of inflammatory cytokines



PPARγ non agonist (SR1664) inhibited inflammatory cytokines as well as TZD
Different scaffold SR1931 did not - compound is a weak binding non-agonist

LPS- RAW264.7 cells

- preincubation with compounds for 18 hrs
- then LPS stimulation for 6 hrs

PTM status impacts PPARG function: How?



TNF-α **ΡΡΑ**_Υ^{WT} PPARγ^{S273A} Ł NT TNF α NT TNF α Thrap3 M.W. (kDa) $IP : PPAR\gamma$ PPARγ 191 MS ID \leftarrow PPAR γ 97 64 Thrap3 - PPARγ **Total cell lysates** 51 39 SR1664/TNF-α MRL24/TNF- α Rosi/TNF-α 28 TNF-α 14 Ę ← Thrap3 **IP: PPARγ** PPARγ PPARγ **Total cell lysates** Thrap3

Thrap3 is a factor that binds to PPARG when S273 is phosphorylated. Functional studies ongoing.

SR1664 POC Thrap3 CoRep P Repression of a subset of PPARG target genes CoAct(x) PPRE CoA target gene AF2 P + SR1664 Ľ Thrap3` CoA AF1 Activation (agonism) of a CoRep subset of PPARG target genes Ligand – PTM **PTM** – co-regulator CoAct(x) PPRE target gene

Crystal Structure of PPARG:SR1664



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SR1664 structure = grey *PDB:2PRG

Structure of Enantiomers SR1663 & SR1664







HDX Analysis of Proteins



- Protein conformational mobility influences rate of amide H atoms to exchange with solvent D atoms.
- Solution based fully automated system; LC-MS LTQ-Orbitrap with ETD to combine bottom-up HDX with ETD sub-localization.

Differential HDX Work Flow

Incubation with D₂O buffer



= Deuterium

Chalmers et al JBT 2007 Chalmers et al Exp Rev Prot 2011

HDX Profiling of Ligands

| Name | Row | Sequence | 9034 | AMG131 | 1664 | 1824 | 1708 | GW9662 | 1665 | SR129 | 1828 | 1931 | Rosi | Pio | MRL20 | MRL24 | 855881 |
|--------------|-----|----------------------------|---------|---------|---------------|---------|---------|---------|---------|---------|---------------|---------|---------|---------------------|---------|----------|-----------|
| 239-250 (+3) | 32 | LRALAKHLYDSY | 0 (1) | -1 (1) | 0 (1) | 0 (1) | 0 (1) | 1 (1) | 1 (1) | 0 (1) | 1 (2) | -1 (1) | 0 (1) | 0 (1) | N/A | 1 (1) | 0 (1) |
| 240-249 (+3) | 42 | RALAKHLYDS | 1 (1) | 0 (1) | 0 (1) | 0 (1) | 1 (2) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | -3 (2) | 0 (0) | 0 (1) | 1 (0) | 0 (0) | 0 (1) |
| 240-250 (+3) | 30 | RALAKHLYDSY | 2 (2) | -1 (2) | 0 (1) | 0 (1) | 0 (1) | 0 (0) | 0 (2) | -1 (1) | 0 (1) | -1 (2) | 1 (1) | 0 (1) | 0 (1) | 1 (0) | -1 (1) |
| 251-265 (+3) | 37 | IKSFPLTKAKARAIL | -15 (3) | 3 (3) | -8 (3) | -7 (2) | -9 (3) | 11 (2) | -6 (2) | 1 (3) | -14 (3) | -4 (2) | -10 (2) | -10 (2) | -4 (1) | -5 (2) | -7 (3) |
| 266-284 (+3) | 54 | TGKTTDKSPFVIYDMNSLM | -2 (4) | -3 (4) | 1 (3) | -1 (3) | 1 (3) | 2 (2) | 0 (2) | 3 (2) | -4 (3) | -4 (2) | -4 (2) | -5 (3) | N/A | -6 (2) | 3 (3) |
| 266-280 (+3) | 38 | TGKTTDKSPFVIYDM | N/A | -3 (3) | 0 (3) | -1 (3) | 1 (3) | 2 (2) | -2 (3) | 3 (2) | -6 (3) | -6 (2) | -5 (2) | -6 (2) | 1 (1) | -5 (2) | 3 (3) |
| 285-306 (+3) | 59 | MGEDKIKFKHITPLQEQSKEVA | 0 (3) | 1 (3) | 1 (3) | 0 (3) | 1 (2) | 1 (2) | 1 (3) | 1 (3) | -1 (6) | 1 (2) | 0 (3) | 1 (3) | 0 (2) | -1 (3) | 1 (3) |
| 285-304 (+3) | 43 | MGEDKIKFKHITPLQEQSKE | 1 (3) | 1 (3) | 1 (3) | -2 (2) | 1 (3) | 1 (2) | 1 (2) | 2 (3) | 0 (6) | 1 (2) | 0 (2) | 1 (3) | 1 (2) | -1 (2) | N/A |
| 289-306 (+3) | 25 | KIKFKHITPLQEQSKEVA | 0 (3) | 1 (4) | 1 (4) | 0 (3) | 1 (3) | 2 (3) | 1 (3) | 2 (3) | -1 (7) | 1 (3) | 1 (3) | 1 (3) | -1 (2) | -1 (3) | 1 (3) |
| 307-314 (+2) | 8 | IRIFQGCQ | -76 (4) | -94 (2) | -37 (4) | -58 (3) | -44 (4) | N/A | -8 (4) | -16 (5) | N/A | -16 (3) | -67 (5) | -17 (5) | -66 (2) | -80 (2) | -9 (4) |
| 315-319 (+2) | 51 | FRSVE | N/A | N/A | -32 (4) | -59 (3) | N/A | -3 (4) | N/A | N/A | N/A | -26 (3) | -53 (7) | N/A | N/A | -82 (3) | N/A |
| 316-337 (+3) | 15 | RSVEAVQEITEYAKSIPGEVNL | -23 (1) | -22 (2) | -15 (1) | -17 (1) | -15 (2) | -6 (1) | -7 (1) | -14 (2) | N/A | N/A | -19 (1) | -18 (2) | N/A | -20 (1) | -16 (2) |
| 320-326 (+1) | 9 | AVQEITE | -26 (1) | -20 (2) | -19 (2) | -20 (2) | -17 (2) | -11 (2) | -11 (1) | -22 (2) | -38 (3) | -18 (1) | -22 (1) | -34 (2) | -14 (2) | -27 (1) | -29 (2) |
| 321-320 (+1) | 40 | VAKSIDGE | 0.00 | -21 (2) | -16 (1) | -10 (2) | -16 (3) | -11(1) | -11 (2) | -19 (2) | -30 (3) | -10 (2) | -10 (1) | -37 (2) | -12 (1) | -20 (2) | -51 (2) |
| 327-337 (+2) | 41 | YAKSIPGEVNI | 1 (1) | 1 (3) | 0 (0) | 0 (1) | 0 (0) | 0 (1) | 0 (0) | 1 (1) | | | | $ \longrightarrow $ | | | 40 |
| 335 339 (+1) | 36 | VNLDI | NIA | N/A | N/A | 1(2) | N/A | N/A | N/A | N/A | | | | | | | |
| 338-345 (+1) | 2 | DI NDOVTI | -1 (2) | 0 (1) | 0 (1) | .1 (1) | 1 (2) | 1 (1) | 1 (1) | 0 (1) | Ê. | | | | | | 30 |
| 338-346 (+2) | 64 | DLNDQVTLL | N/A | 0 (1) | N/A | 0 (1) | N/A | 0 (1) | N/A | 0 (1) | | | | | | | |
| 346-352 (+2) | 45 | LKYGVHE | -1 (1) | -3 (1) | -1 (1) | -2 (1) | -2 (1) | -2 (2) | -1 (1) | -1 (1) | | | | | | | 20 |
| 346-357 (+3) | 35 | LKYGVHEIIYTM | 0 (1) | -1 (0) | 0 (1) | -1 (1) | -1 (1) | 0 (1) | 0 (1) | -1 (1) | | | | | | | |
| 346-355 (+2) | 53 | LKYGVHEIIY | -1 (0) | -2 (1) | -1 (1) | -1 (0) | -1 (0) | 0 (0) | -1 (1) | -1 (0) | | | | | | | 10 |
| 358-368 (+2) | 39 | LASLMNKDGVL | -5 (2) | -6 (2) | -3 (1) | -4 (1) | -5 (3) | -2 (1) | -5 (1) | -1 (2) | AV | | | | | | 10 |
| 359-368 (+2) | 52 | ASLMNKDGVL | -5 (2) | -7 (2) | -4 (1) | -4 (1) | -5 (2) | -1 (1) | -5 (1) | -2 (3) | | | | | | | - |
| 362-368 (+2) | 5 | MNKDGVL | -6 (2) | -7 (2) | -4 (1) | -4 (2) | -6 (1) | -1 (1) | -5 (1) | 0 (2) | | | | | | \ | 5 |
| 369-391 (+3) | 21 | ISEGQGFMTREFLKSLRKPFGDF | -15 (3) | -19 (2) | -9 (2) | -8 (3) | -10 (2) | -3 (2) | -7 (2) | -4 (3) | | n | | | |) | 1.0101000 |
| 369-379 (+2) | 34 | ISEGQGFMTRE | -24 (3) | -23 (3) | -16 (3) | -16 (3) | -17 (2) | 0 (3) | -12 (3) | -3 (4) | | | | V | | | ns |
| 369-381 (+2) | 31 | ISEGQGFMTREFL | -18 (4) | -17 (3) | -12 (2) | -13 (2) | -13 (2) | 0 (2) | -9 (2) | -3 (3) | | | | Show | | | |
| 380-390 (+2) | 11 | FLKSLRKPFGD | -3 (1) | 0 (2) | -3 (2) | -3 (2) | -4 (3) | -2 (1) | 1 (3) | -2 (1) | | | | | | | -5 |
| 380-398 (+3) | 55 | FLKSLRKPFGDFMEPKFEF | -16 (2) | -19 (2) | -5 (2) | -6 (2) | -8 (2) | -3 (2) | -5 (1) | 1 (2) | | | | | | | -10 |
| 384-391 (+2) | 44 | LRKPFGDF | -17 (3) | -29 (3) | -2 (3) | -3 (2) | -3 (4) | -4 (2) | -5 (3) | N/A | ř. | | | | 2 | | |
| 384-398 (+3) | 10 | LRKPFGDFMEPKFEF | -18 (2) | -21 (2) | -5 (2) | -5 (2) | -7 (2) | -3 (2) | -5 (1) | 3 (2) | | | | | | | -20 |
| 399-412 (+2) | 29 | AVKENAL | -4 (1) | -6 (1) | 0 (1) | 1 (1) | -2 (1) | 1 (1) | -3 (1) | -2 (1) | 0 | | | | | | |
| 399-403 (+2) | 1 | | -0 (1) | -11 (2) | -2 (1) | -1 (2) | -4 (1) | -2 (1) | -3 (1) | -3 (1) | | | | | | | -30 |
| 405-412 (+1) | 22 | | -1 (1) | -1 (1) | 1 (1) | 2(1) | 1 (1) | 2 (1) | 1 (1) | 2(1) | -1 | | | | | 7 | |
| 418 441 (+3) | 28 | | -1 (1) | 1 (1) | 0 (1) | 1(1) | 0 (1) | 1 (1) | 0 (1) | 0 (1) | | | | 1000 | | | -40 |
| 418-429 (+2) | 12 | VIII SGDRPGLI | 2(1) | 2 (2) | -1 (1) | .1 (2) | -3 (2) | 1(1) | 0 (1) | -2 (1) | | | | | | | |
| 418-436 (+3) | 19 | VIII SGDRPGI I NVKPIED | -2 (1) | -1 (2) | -1 (2) | -2 (2) | -2 (1) | 0 (1) | 0 (2) | 0 (1) | | | | | | | N/A |
| 419-441 (+3) | 13 | IIL SGDRPGLLNVKPIEDIQDNL | -5 (2) | -2 (2) | 0 (1) | -1 (1) | -1 (1) | 0 (1) | 0 (1) | 0 (2) | | | | | | | 0 (1) |
| 419-429 (+2) | 49 | IIL SGDRPGLL | -2 (2) | -1 (2) | 0 (1) | -2 (2) | -2 (1) | -1 (1) | 0 (1) | -1 (1) | | | | | | | 1 (2) |
| 419-436 (+3) | 24 | IIL SGDRPGLLNVKPIED | -2 (2) | -2 (2) | 0 (1) | -1 (1) | -1 (1) | -1 (1) | 0 (1) | -1 (1) | | | | | | (| 0 (1) |
| 419-435 (+3) | 20 | IIL SGDRPGLLNVKPIE | -2 (1) | -2 (2) | 0 (1) | -2 (1) | -1 (1) | 0 (1) | 0 (1) | -1 (1) | | | | | | J | 1 (1) |
| 430-436 (+2) | 33 | NVKPIED | N/A | -3 (4) | 4 (4) | 0 (1) | N/A | 1 (1) | -1 (3) | 1 (1) | | | | | | > | 1 (1) |
| 430-444 (+2) | 16 | NVKPIEDIQDNLLQA | 0 (0) | 0 (0) | 0 (0) | 0 (1) | 0 (0) | 0 (1) | 1 (0) | 0 (0) | | | | | | | 0 (1) |
| 430-441 (+2) | 14 | NVKPIEDIQDNL | 0 (1) | -1 (1) | 1 (2) | 0 (1) | 1 (1) | 0 (1) | 0 (1) | 0 (1) | | | | | | | 0 (1) |
| 437-442 (+1) | 40 | IQDNLL | 0 (0) | -1 (0) | 1 (0) | 1 (1) | 0 (1) | 1 (1) | 0 (0) | 0 (1) | | - | | | | | -1 (1) |
| 445-459 (+3) | 3 | LELQLKLNHPESSQL | 0 (1) | 0 (2) | 0 (1) | 0 (1) | 1 (1) | 1 (1) | 1 (2) | 1 (2) | | 1 1 | | | - | | 1 (1) |
| 446-459 (+2) | 58 | ELQLKLNHPESSQL | -1 (2) | 2 (2) | 2 (2) | 1 (1) | 1 (2) | 1 (1) | 1 (1) | 0 (2) | | | | | | | 1 (1) |
| 448-459 (+2) | 10 | QLKLNHPESSQL | -1 (2) | 1 (3) | 0 (3) | 1 (2) | 0 (4) | 1 (1) | 1 (2) | 10 | | | | | | | 1 (2) |
| 449-439 (+2) | 40 | KINHPESSOL | -2 (2) | -2 (3) | 2 (3) | 0 (2) | 1 (2) | N/A | 1 (2) | | | | | | | | N/A |
| 450-459 (+2) | 10 | CAKILOKMTDI | -1 (5) | -1 (4) | 2 (3) | 0 (3) | 2 (1) | 1 (1) | 1 (5) | | 2 (2) | 2 (4) | 2 (1) | 1 (1) | A (4) | 1 (1) | 1 (3) |
| 460 472 (+2) | 57 | EAKLLOKMIDL | 2 (1) | -1 (2) | -1 (5) N/A | -1 (1) | -2 (1) | 1 (2) | 0 (1) | 2(1) | -2 (2) N/A | 3(1) | -2 (1) | 2 (1) | 3 (0) | 0 (1) | -2 (2) |
| 464 472 (+3) | 30 | LOKMIDLEO | 1(2) | 1 (2) | NUA | 1(2) | 0 (2) | 0 (1) | | 2(2) | N/A | 2 (2) | 6(1) | -2 (1) | N/A | 0 (1) | 5(2) |
| 464.470 (+2) | 27 | LOKMTDI | 0 (1) | 0 (2) | 0 (2) | 2 (1) | 1 (2) | 2 (1) | | 2(1) | 0 (2) | A (2) | 3 (1) | 2(1) | 6 (1) | 1 (1) | 3(1) |
| 471.480 (+3) | 6 | ROIVTEHVOI | -19 (2) | -28 (2) | 4(3) | 5 (3) | 4 (2) | 5(2) | 511 | 4(2) | 2 (3) | 0(1) | -36 (1) | -31 (2) | N/A | -11 (2) | -13 (2) |
| 471-476 (+2) | 60 | ROIVTE | -19 (2) | -24 (2 | 6 (2) | 7 (2) | 5 (2) | 9 (2) | 4 2) | 0 (3) | N/A | 1(2) | -32 (1) | -28 (2) | N/A | 0 (2) | -11 (3) |
| 481-497 (+3) | 17 | LOVIKKTETDMSLHPLL | -11 (4) | -4 (4) | 1 (4) | -1 (4) | -1 (3) | 2 (3) | 8 (3) | 1 (3) | -1(7) | -1 (2) | -7 (3) | -7 (3) | -17 (2) | -6 (3) | 0 (3) |
| 481-499 (+3) | 4 | LQVIKKTETDMSLHPLLQE | -12 (3) | -3 (4) | 0 (4) | -2 (3) | -1 (3) | 5 (3) | -1 (3) | 1 (3) | -1 (5) | -2 (2) | -8 (2) | -7 (3) | -14 (2) | -6 (3) | -2 (6) |
| 481-491 (+2) | 61 | LQVIKKTETDM | -11 (6) | -9 (5) | 2 (4) | 1 (4) | -2 (3) | 2 (3) | -2 (4) | -1 (3) | -2 (6) | -1 (2) | -5 (4) | -9 (3) | -23 (2) | -8 (3) | 3 (5) |
| 484-497 (+3) | 0 | IKKTETDMSLHPLL | -7 (3) | 1 (4) | 1 (3) | -1 (3) | 0 (2) | 2 (2) | 1 (3) | 1 (3) | -1 (6) | 1 (2) | -5 (3) | -1 (3) | N/A | -1 (2) | -2 (3) |
| 484-491 (+2) | 56 | IKKTETDM | N/A | N/A | N/A | -1 (4) | N/A | 3 (3) | N/A | N/A | N/A | N/A | 1 (3) | N/A | N/A | N/A | N/A |
| 492-505 (+2) | 47 | SLHPLLQEIYKDLY | -20 (4) | -2 (5) | 3 (5) | -2 (3) | 2 (3) | 1 (3) | 0 (4) | -3 (5) | 0 (10) | N/A | -26 (3) | -9 (3) | -13 (2) | N/A | -4 (5) |
| 492-499 (+2) | 56 | SLHPLLQE | N/A | -12 (5) | -1 (4) | -9 (6) | 0 (5) | 6 (5) | N/A | -2 (5) | -2 (6) | N/A | -18 (2) | N/A | -12 (3) | N/A | -12 (5) |
| 494-505 (+2) | 26 | HPLLQEIYKDLY | -19 (4) | 0 (5) | 1 (5) | 0 (5) | -2 (3) | 1 (3) | N/A | -1 (6) | 2 (7) | 0 (3) | -26 (4) | -10 (6) | -14 (2) | N/A | -8 (4) |
| 498-505 (+1) | 23 | QEIYKDLY | -15 (3) | 2 (2) | 5 (5) | 1 (3) | 1 (4) | 3 (6) | 0 (4) | 1 (5) | 1 (6) | 1 (3) | -27 (3) | -10 (4) | -12 (1) | -6 (3) | -7 (4) |

HDX Differentiates Functionally Distinct Enantiomers SR1663 & SR1664



-40 -50 SR1664

80nM

Not active (0%)

HDX Differentiates Functionally Distinct Enantiomers SR1663 & SR1664





| | | 750 | /00 | -50 |
|---------------|-------------------------|------|--------------|----------|
| Compound | IC50 (binding affinity) | EC50 | (PPRE) (% | relative |
| | | to | rosiglitaz | one) |
| rosiglitazone | 18nM | | 7.4nM (100 | %) |
| SR1663 | 2nM | | 20nM (23% | %) |
| SR1664 | 80nM | Ν | lot active (| 0%) |

HDX Differentiates Functionally Distinct Enantiomers SR1663 & SR1664



Ligand Size and Contacts are Critical

SR2536 Reveals Cause of H12 Stabilization – 'Flipping'



SR1664 Mechanism of Action







PPARG Co-Regulator Binding



SR1664 Mechanism of Action



Differing Effects of PPARy Ligands on RXR α



Summary of Agonism SAR

• PPARG Paradigm has changed to development of 'Nonagonist' ligands that modulate PTMs.

• HDX allows us to differentiate SR1663 & SR1664 which look identical in static crystal structure but are functionally diverse.

• Degree of H12 stabilization measured by HDX correlates with activity, H3 stabilization correlates with affinity.

• Rosi & SR1664 have different effects on RXRα dynamics which may indicate altered heterodimer affinity/interaction.

Summary of SR1664

- .. is a potent binder to PPARG Kd similar to rosiglitazone.
- .. lacks classical AF2 driven agonism completely inactive in PPRE:Luc assays and no alteration of agonist genes *in vivo*.
- ... blocks S273-P in cells and *in vivo* and is anti-diabetic with improved AE profile versus TZDs.
- .. is an antagonist of natural ligand but agonist of S273-P repressed gene set. The compound disrupts receptor and co-receptor (RXR) conformational dynamics interfering with binding of CoA or release of CoR.
- .. has poor PK and solubility. Some formulations of the compound are toxic.
- We have SAR on partial agonist to non-agonists with over 20 unique nonagonist compounds to date. We have some insight into the molecular mechanism.
- Questions will non-agonists have similar anti-inflammatory properties as TZDs *in vivo*? TZDs brown fat, and partial and non-agonists do not – will this limit their efficacy?





Rosiglitazone and SR10171 (20mg/kg P.O.) in DIO model









BODY WEIGHT PERCENTAGE DATA: MiniSpec NMR analyzer

Summary

PPARG – Demonstrated that PO3 of PPARG at S273 controls a subset of target genes that are dysregulated in obesity (*Nature* 2010). This led to the discovery of novel modulators that bind to PPARG without inducing AF2-dependent agonism (passive antagonism). These compounds block S273-P and they are efficacious in diabetic mice (*Nature* 2011). But will this be enough for robust efficacy, and can this scaffold be optimized for appropriate pharmaceutical properties?





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<u>Funding of Work Presented</u>: NIH - GM084041, U54MH084512, RC4DK090861 SR2278/SR10171 study funded by Ember Therapeutics

