PPARδ Modulators Improve Mitochondrial Function: a Potential Treatment for DMD

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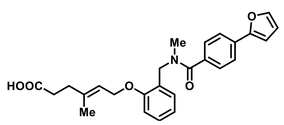
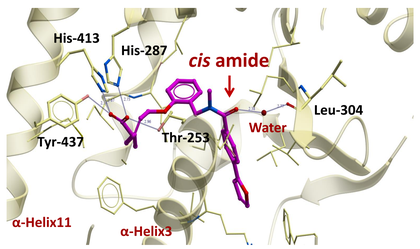
Abstract

Benzamides such **1**, have been reported as orally bioavailable PPARδ modulators with improved safety profile in rodents.[1,2] X-ray structure of **1** bound to the ligand binding domain (LBD) of PPARδ, revealed that the amide moiety exists in thermodynamically unfavorable *cis*-amide conformation.[3] Among the heterocyclic analogs that were tried as isosteric replacements of the *cis*-amide, imidazoles emerged as highly potent and selective modulators of PPARδ. Further exploration of SAR helped optimize the pharmacokinetic parameters. The lead compound, **MA-0204** increased PPARδ target gene expression and improved mitochondrial functions in DMD patient cells suggesting a role for the selective PPARδ modulator as a treatment of DMD.

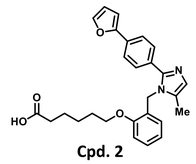


Cpd. 1

PPARδ EC_{50} = 37 nM
PPARα EC_{50} = 46,000 nM

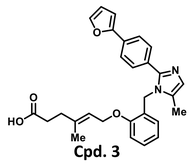


Selected SAR and i.v. PK parameters



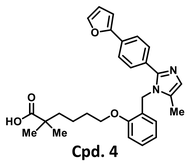
Cpd. 2

PPARδ EC_{50} = 0.7 nM
PPARα EC_{50} = 6,900 nM
CL = 270 mL/min/kg
AUC = 180 ng*h/mL



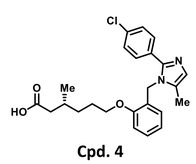
Cpd. 3

PPARδ EC_{50} = 1.0 nM
PPARα EC_{50} = 1230 nM
CL = 160 mL/min/kg
AUC = 310 ng*h/mL



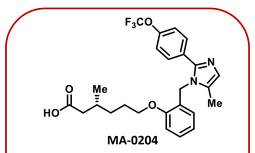
Cpd. 4

PPARδ EC_{50} = 4.5 nM
PPARα EC_{50} = ND
CL = 120 mL/min/kg
AUC = 400 ng*h/mL



Cpd. 4

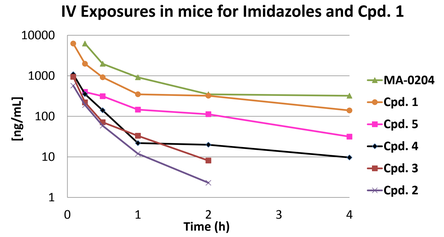
PPARδ EC_{50} = 5.5 nM
PPARα EC_{50} >100,000 nM
CL = 22 mL/min/kg
AUC = 750 ng*h/mL



MA-0204

PPARδ EC_{50} = 0.4 nM
PPARα EC_{50} = 6,990 nM
$T_{1/2}$ = 2.7 h; CL = 27 mL/min/kg
AUC = 1,200 ng*h/mL

IV Exposures in mice for Imidazoles and Cpd. 1

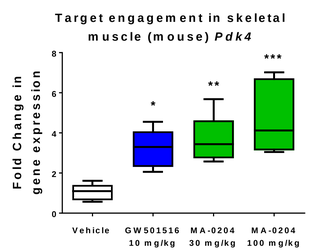


[a]Transactivation assay with human PPARs; For all compounds EC_{50} for PPARγ >100,000 nM;
[B] I.V. PK in Male CD-1 mice (3 mpk dose in 2% DMA, 20% HPßCD in water).

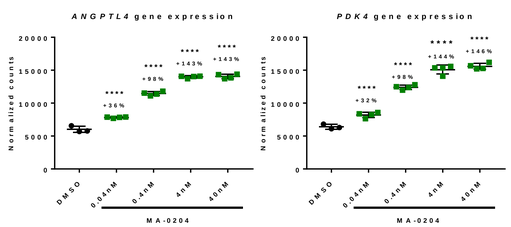
In vivo and *ex-vivo* Target Engagement

MA-0204 upregulates gene expression of target gene after single oral administration in mice (right panel)

MA-0204 upregulates gene expression of target genes in myoblasts derived from a 17-month old DMD patient (panel below).

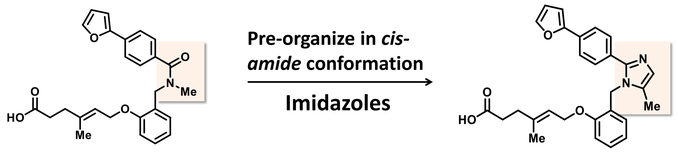


Target engagement in myoblasts from patient



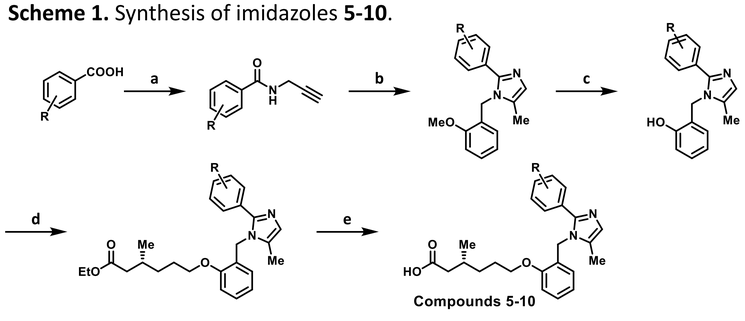
MA-0204 was added to the culture media for 48 hours prior to RNA isolation.
% change values represent difference in median value from DMSO vehicle control.

From Benzamides to Imidazoles



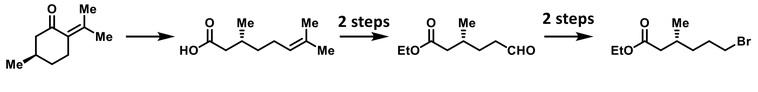
- *Cis*-amide conformation is uncommon even in peptides.[4]
- *Cis* amide **1** energetically unfavorable by about 1.3 kcal/mole than the *trans* amide.[5]

Scheme 1. Synthesis of imidazoles **5-10**.

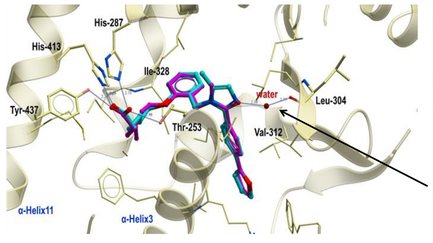


Reagents and conditions: a) Propargyl amine, EDCI.HCl, HOBt, Et_3N, DMF, RT, 12 h; b) 2-Methoxybenzyl amine, $Zn(OTf)_2$, toluene, 120°C, 12 h; c) BBr_3, DCM, RT, 2 h; d) Ethyl (*R*)-6-bromo-3-methylhexanoate, KO^{t}Bu, DMF, RT, 2 h; e) $LiOH.H_2O$, THF, EtOH, H_2O, RT, 12 h.

Scheme 2. Synthesis of ethyl (*R*)-6-bromo-3-methylhexanoate



X-ray Structures: Amide (Cpd. **1**) and Imidazole (Cpd. **2**)



PPARδ LBD-bound x-ray structures for imidazole 2 (cyan) and benzamide 1 (magenta)

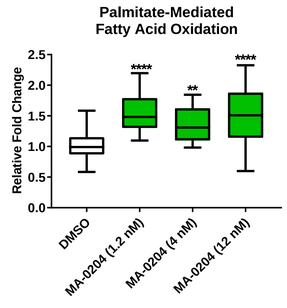
- Similar binding modes and interactions with PPARδ observed
- Water mediated interactions retained for imidazole **2**

MA-0204: Rodent PPARδ Potency and PK

Assay	Results
Mouse PPARδ EC_{50}	7.9 nM
Rat PPARδ EC_{50}	10 nM
Mouse PK (1 mpk i.v. and 10 mpk p.o.)	$t_{1/2}$ = 2.7h ; V_{ss} = 5.8 L/kg ; AUC = 630 ng.h/mL; C_{max} = 510 ng/mL; %F = 42
Rat PK (1 mpk i.v. and 3 mpk p.o.)	$t_{1/2}$ = 3.3h ; V_{ss} = 1.8/kg ; AUC = 4900 ng.h/mL; C_{max} = 1100 ng/mL; %F = 90

MA-0204 is selective (IC/EC_{50} >10 μM) in a panel of >40 receptors (including AR, ER, GR and PR) and transporters.

Effect on Fatty Acid Oxidation



Defects in mitochondrial respiration[6] are reported in Duchenne muscular dystrophy (DMD). We tested fatty acid oxidation (FAO) in muscle cells derived from primary myoblasts isolated from a 17-month old DMD patient using Seahorse Respiration assay. MA-0204 restores defective FAO in cells after 48 hr of treatment. Consistent with an improvement in energetics, MA-0204 also increases the ATP/ADP ratio in mdx muscle cells (data not shown).[7]

Taken together, these results support further investigation of MA-0204 as a treatment for DMD.

References

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2. Lagu, B. et al. "Highly selective peroxisome proliferator-activated receptor δ (PPARδ) modulator demonstrates improved safety profile compared to GW501516" *Bioorg. Med. Chem. Lett.* **2018**, *28*, 533.
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