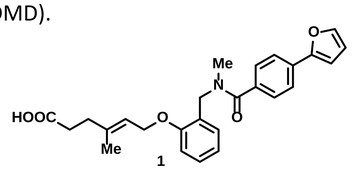
PPARδ Modulators Improve Mitochondrial Function: a Potential Treatment for DMD

Bharat Lagu*#, Arthur F. Kluge#, Effie Tozzo#, Eric Bell#, Peter Dwyer#, Matthew Goddeeris#, Ross Fredenburg#, Ramesh Senaiar¶, Mahaboobi Jaleel¶, Sunil K. Panigrahi¶, Narasimha R. Krishnamurthy,¶ Anirudha Lakshminarasimhan, ¶ Nirbhay K. Tiwari ,¶ Taisuke Takahashi,^ and Michael A. Patane#

#Mitobridge, Inc. Cambridge, MA 02138; ¶Aurigene Discovery Technologies Ltd, Hyderabad, India; ^Astellas Pharma., Tsukuba, Japan

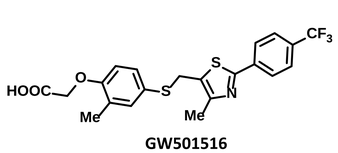
Abstract

Evans and co-workers have recently reported benzamides as highly selective PPARδ modulators.[1] We have modified the structure of these benzamides to identify compound **1** with significantly improved PK profile while maintaining potency and selectivity for PPARδ[2]. We now report an imidazole series of highly potent and selective modulators of PPARδ designed based on the x-ray structure of **1** bound to the ligand binding domain (LBD) of PPARδ. Further refinements of the structure improved the pharmacokinetic properties. The lead compound, MA-0204 increased PPARδ target gene expression in skeletal muscle and improved mitochondrial defects in the mdx mouse myoblasts suggesting a role for MA-0204 for treatment of Duchenne muscular dystrophy (DMD).



1

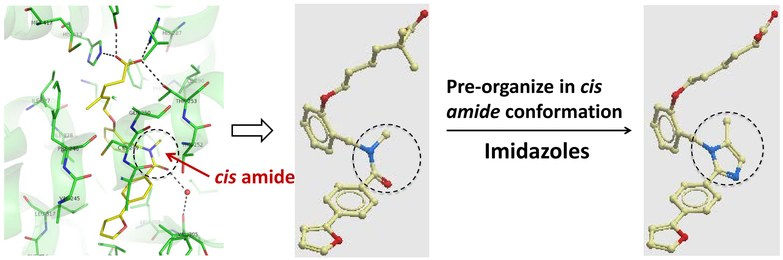
PPARδ EC_{50} = 37 nM
PPARα EC_{50} = 46,000 nM



GW501516

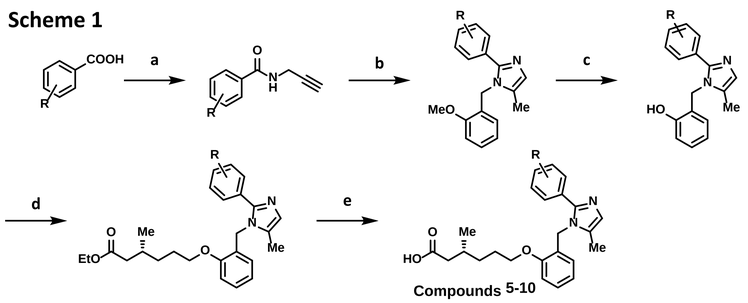
PPARδ EC_{50} = 2.0 nM
PPARα EC_{50} = 6,600 nM

Ligand Bound X-ray Structure



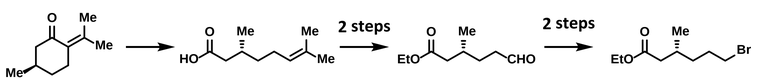
Cis amide **1** energetically unfavorable by about 1.3 kcal/mole than *trans* amide.[3]

Scheme 1

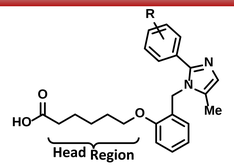


Reagents and conditions: a) Propargyl amine, EDCI.HCl, HOBt, Et_3N, DMF, RT, 12h; b) 2-Methoxybenzyl amine, $Zn(OTf)_2$, toluene, 120°C, 12h; c) BBr_3, DCM, RT, 2h; d) Ethyl (*R*)-6-bromo-3-methylhexanoate, KO^{t}Bu, DMF, RT, 2 h; e) LiOH.H_2O, THF, EtOH, H_2O, RT, 12h.

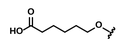
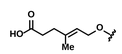
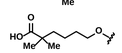
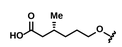
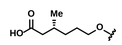
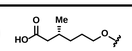
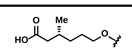
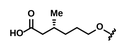
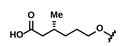
Scheme 2. Synthesis of ethyl (*R*)-6-bromo-3-methylhexanoate



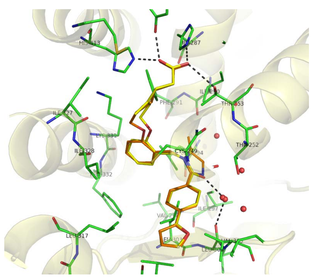
SAR and i.v. PK parameters



Compounds 2-10

Compound	Head Region Modification	R	PPARδ EC_{50} (nM)[a]	PPARα EC_{50} (nM)[a]	CL[b] mg/mL/kg	AUC[b] ng.h/mL
2		4- 	0.7	6,900	270	180
3		4- 	1.0	1,200	160	310
4		4- 	4.5	ND	120	390
5		4- 	3.7	>10,000	73	650
6		4-$OCHCF_2$	14	15,000	ND	ND
7 (MA-204)		**4-OCF_3**	**0.4**	**6,900**	**27**	**1200**
8		4-Cl	5.0	>100,000	22	750
9		4-Me	19	78,000	ND	ND
10		4-Cl-3-F	4.3	69,000	38	440

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



PPARδ LBD-bound x-ray structures for imidazole 2 and benzamide 1

- Similar binding modes and interactions with PPARδ observed
- Water mediated interactions retained for both ligands

MA-0204: Rodent PPARδ Potency and PK

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

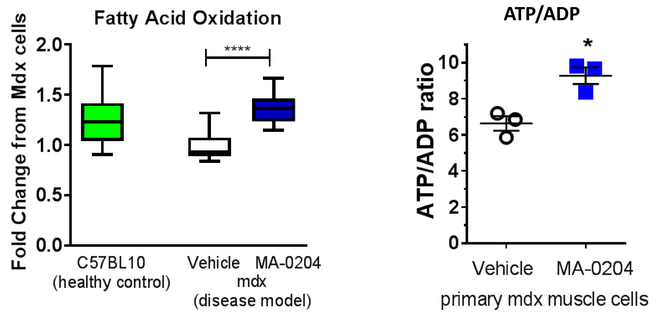
MA-0204 is selective (IC/EC_{50} >10 μM) in a panel of >40 receptors (including AR, ER, GR and PR) and transporters.

Gene Regulation: Undifferentiated human muscle cells were treated at EC_{50} with MA-0204 or GW501516 for 24 hours and evaluated for gene regulation by RNAseq

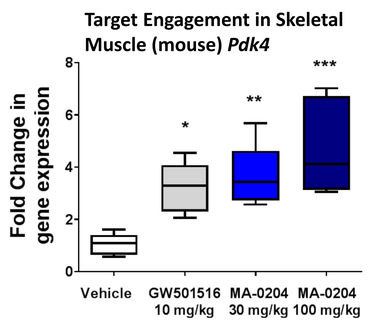
Top results of functional KEGG Annotation of common target genes:

- Mitochondrion
- Transit Peptide
- Mitochondrion inner membrane
- Fatty acid metabolism
- Lipid metabolism

Fatty Acid Oxidation and Target Engagement



Defects in mitochondrial respiration[4] are reported in Duchenne muscular dystrophy (DMD). We tested fatty acid oxidation (FAO) in muscle cells derived from a DMD mouse model (referred to as ***mdx***) by Seahorse Respiration assay. MA-0204 (@800 nM) restores defective FAO in cells after 48 hours of treatment. Consistent with an improvement in energetics, MA-0204 increases the ATP/ADP ratio in mdx muscle cells[5].



Oral administration of MA-0204 results in an increase in PPARδ target gene, *Pdk4* (pyruvate dehydrogenase kinase 4,) in skeletal muscle. These results support further investigation of MA-0204 as a treatment for DMD.

References

[1] Wu, C. et al. "Structural basis for specific ligation of the peroxisome proliferator-activated receptor δ. *PNAS* Vol 114 (13), E2563-2570 (2017).
[2] Manuscript submitted (Mitobridge)
[3] Molecular mechanics based energy minimization and conformational analysis study using Molsoft/MMFF force field
[4] Schuh RA, et al. *Am J Physiol Regul Integr Comp Physiol.* 302(6):R712-9 (2012).
[5] E. Bell et al. "PPARδ modulation partially restores mitochondrial defects resulting from dystrophin loss of function" Keystone Mitochondrial Symposium (2016)

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