Development of MLX0871, a Potent, Isoform Selective, Orally Available AMPK Activator, Displays Efficacy in Obese and Type 2 Diabetes Models

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ABSTRACT

Introduction & Objectives: AMPK is a αβγ heterotrimeric Ser/Thr kinase that acts as a cellular energy sensor and is activated by upstream enzymes when the cellular ratio of AMP to ATP is elevated due to nutrient deprivation. AMPK activity can be altered due to many physiological factors, such as hormones, cytokines, dietary nutrients, and pathological conditions, such as obesity, chronic inflammation, and type 2 diabetes. AMPK activation can lead to lower hepatic and plasma glucose levels. Thus, AMPK is an attractive target for treating various metabolic diseases.

Methods: 12 isoforms of AMPK in activation mode using radiometric HotSpot™ assay. Glucose Uptake and Western Blot cell-based assays were conducted in human skeletal muscle, upper arm, white preadipocyte, abdomen, and hepatocyte cells. Male C57BL/6 DIO mice aged 20 weeks were fed with a high-fat diet (60 kcal%) for 14 weeks before initiating the experiment. A vehicle group was compared to two dose levels of 10 and 30 mg/kg, dosed orally for the 30-day treatment, with weight loss and serum glucose regulation measured at regular intervals.

Results: MLX0871 and analogs are orally available small molecule AMPK activators that have been shown to stimulate AMPK activity and enhance glucose uptake in human tissues dose-dependently. MLX0871 do not cause any AMPK activation in healthy, obese, and diabetic patient cardiac muscle tissue samples and suggest selectivity to skeletal muscle. MLX compounds demonstrated safety and tissue specificity. Preclinical studies suggest that MLX0871 may have therapeutic potential in the treatment of metabolic disorders such as obesity and type 2 diabetes.

Conclusions: These results suggest that AMPK activators induce insulin modulation and improve oxidative muscle function in obese and type 2 diabetes patient samples. In vitro data supporting studies will be presented, including DIO and db/db efficacy results.

AMPK ISOFORM SPECIFIC DESIGN - RESULTS

1. Domains of AMPK α, β and γ subunits (**Fig. 1**)

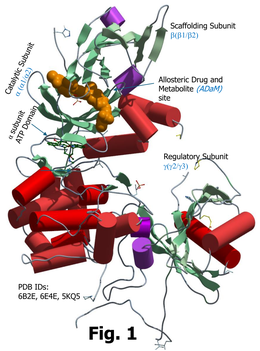


Fig. 1

Table 1: MLX-series & reference AMPK activators selectivity across 12 isoforms

AMPK Isoform	MLX0871 AC_{50} (nM)	PF-06409577 AC_{50} (nM)	MK-8722 AC_{50} (nM)
α1/β1/γ1	>10000	<5.08	10.30
α1/β1/γ2	>10000	<5.08	26.07
α1/β1/γ3	>10000	33.50	367.90
α1/β2/γ1	>10000	5230.00	15.21
α1/β2/γ2	>10000	1831.00	11.46
α1/β2/γ3	>10000	2468.00	35.31
α2/β1/γ1	>10000	18.51	163.80
α2/β1/γ2	5.08	5.08	9.76
α2/β1/γ3	5.29	5.08	15.99
α2/β2/γ1	>10000	8466.00	40.60
α2/β2/γ2	>10000	3972.00	20.53
α2/β2/γ3	>10000	5986.00	10.78

2. AMPK heterotrimer α, β, γ subunit composition in skeletal muscle (**Fig. 2**)

Fig. 2

IN VITRO RESULTS

3. AMPK Isoform Specific Actors Design: Knowledge-based, Fragments/Scaffold-hopping within AMPK crystal structures ADaM, eADME, synthesis, and testing of over 60 plus AMPK activators led to the selection of MLX0871 (**Fig. 3**)

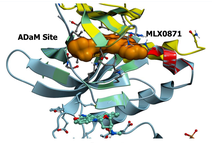


Fig. 3

4. KINOMEscan panel of 468 Kinases Selectivity: MLX0871 tested at 10 μM concentration (**Fig. 4**)

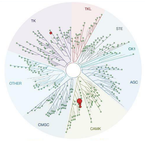


Fig. 4

5. Effect of AMPK activator MLX0871 on HSKMC-Human Skeletal Myoblasts Cells - Glucose Uptake Assay: MLX0871 dose dependently activates insulin in human SKMC, Preadipose and Hepatocyte cells (**Figs. A-E**)

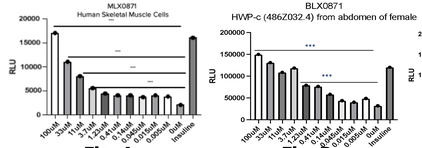


Fig. A

Fig. B

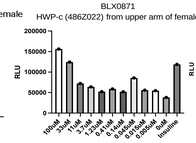


Fig. C

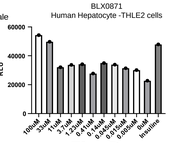


Fig. D

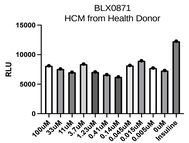


Fig. E

6. Effect of AMPK activator MLX0871 on insulin stimulation in Human Skeletal Myoblasts and HWP cells. The differentiation of Skeletal Myoblast into Myotubes for 48h culturing and the Western Blot experiments results shown (**Figs. F-G**)

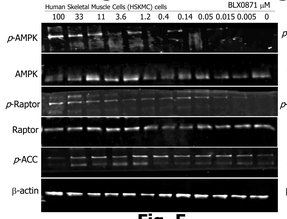


Fig. F

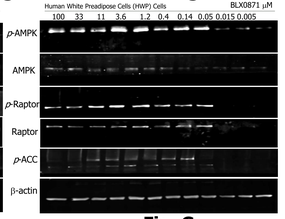


Fig. G

IN VITRO ADME AND IN VIVO PK RESULTS

7. Safety Secondary Pharmacology:

7.1 SelectScreen™ P450 profiling for the assessment of P450 isozyme inhibition by MLX0871: The inhibition effect of MLX0871 IC_{50s} were assessed against 5 P450 isozymes.

P450 Isozymes	MLX0871
1A2	>100 μM
2C19	63 μM
2C9	18.5 μM
2D6	>100 μM
3A4	>100 μM

Study Description	MLX0871
hERG [IC_{50} μM]	39000 nM

7.2 SelectScreen™ Biochemical hERG Activity: The Predictor™ hERG Fluorescence Polarization Assay Kit was used to perform hERG channel biochemical binding studies in the absence of radioligand. MLX0871 hERG activity found to be low μM.

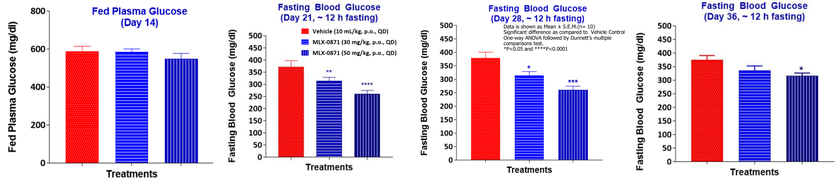
7.3 eurofins/Cerep Safety78Panel Assay on MLX0871: Safety 78 panel assays in the SafetyScan E/IC_{50} utilizing the Path Hunter enzyme fragment complementation (EFC), FLIPR based cellular screening on our lead MLX0871. No significant off target toxicity noted.

8. MLX0871 Pharmacokinetics in Mice, and Rats (Unformulated, dissolved in H_2O):

Table 2	Male SA Mice		Wistar Rats	
Parameters	G1/IV	G2/PO	G1/IV	G2/PO
T_{max} (h)	NA	1.00	NA	1.0
C_0/C_{max}(ng/mL)	4100.67	1157.35	16327.94	NA
AUC_{last}(ng.h/mL)	2685,67	3562.33	4681.72	1324,23
AUC_{inf}(ng.h/mL)	2717.19	3598.95	4690.61	1331.49
Cl(mL/min/kg)	6.13	-	3.65	-
Vd (L/kg)	1.62	-	0.45	-
$T_{1/2}$ (h)	3.05	3.97	2.14	0.89
%F	-	26%		6%

MOUSE db/db EFFICACY RESULTS

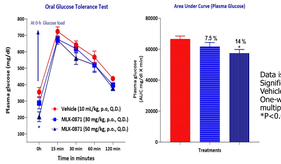
9. Evaluation of Dose Response of MLX0871 on Metabolic Parameters in db/db Mice:



Fed & Fasting Blood Glucose levels measured at days : 14, 21, 28 and 36.

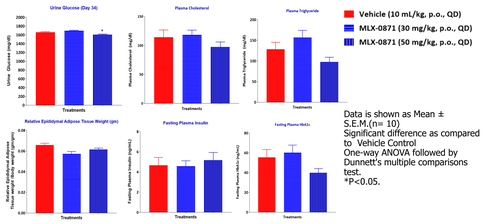
- MXL0871 showed dose depended decrease in fasting blood glucose levels on day 21 ,28 and 36
- MXL0871 (30 & 50 mg/kg, p.o., QD) showed significant decrease in fasting blood glucose on day 21 and 28.
- MXL0871 (50 mg/kg, p.o., QD) showed significant decrease in fasting blood glucose levels on day 36.

10. Oral Glucose Tolerance Test (Day 21): 60 min: Test compound administration, 0 min: Oral glucose load & 0, 15, 30, 60, 120 min for Plasma glucose measurement .



Data is shown as Mean ± S.E.M.(n= 10)
Significant difference as compared to Vehicle Control
One-way ANOVA followed by Dunnett's multiple comparisons test.
*P<0.05.

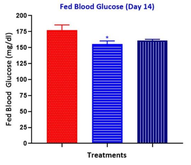
11. Other Key Parameters Tested



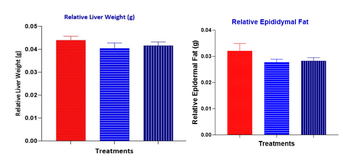
Data is shown as Mean ± S.E.M.(n= 10)
Significant difference as compared to Vehicle Control
One-way ANOVA followed by Dunnett's multiple comparisons test.
*P<0.05.

IN VIVO DIO MOUSE MODEL EFFICACY STUDIES

12. Fed Blood Glucose (Day 14)



13. Liver & Epididymal Fat



Data is shown as Mean ± S.E.M.(n= 10)
Significant difference as compared to G1, Vehicle Control
ANOVA followed by Dunnett's multiple comparisons test.
*P < 0.05,**P < 0.0,***P < 0.001 & ****P < 0.0001

G1-60% HFD + Vehicle Control,p.o.;q.d.
G2-60% HFD + MLX-0871 (30 mg/kg,p.o.;q.d.)
G3-60% HFD + MLX-0871 (50 mg/kg,p.o.;q.d.)

CONCLUSIONS & REFERENCES

- MLX0871 demonstrated isoform specific selectivity for α2/β1/γ2 & α2/β1/γ3.
- MLX0871 exhibited promising in vitro on target AMPK activities.
- MLX0871 is efficacious in vivo db/db & ob/ob mouse models.

Nat Commun **4**, 3017 (2013)
Biochem Pharmacol 153 (2018)
JMC 15 (2016)