DISCOVERY OF CPL207-280CA, AN EFFECTIVE AND SAFE GPR40 AGONIST FOR THE TREATMENT OF TYPE 2 DIABETES.

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INTRODUCTION

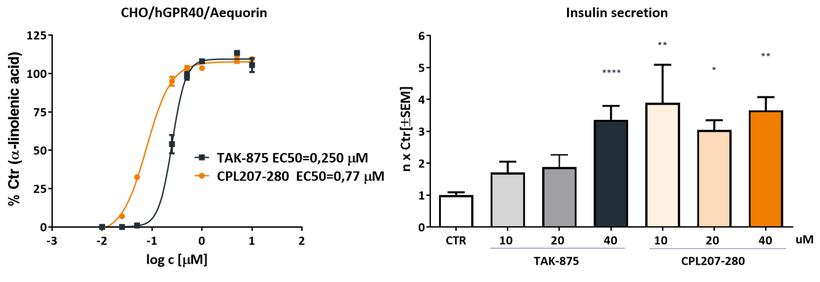
GPR40 receptor (FFAR1) emerged as an antidiabetic target and its activation was proposed as a novel treatment for patients with insufficient blood sugar control or intolerating popular drugs[1]. It belongs to the G-protein coupled receptors family and is highly expressed in pancreatic β cells where mediates free fatty acid-induced insulin secretion. Although several companies were successful in making clinically efficacious GPR40 agonists, none of them proved their compounds safety and finally failed in clinic. The most developed and evaluated in clinical studies was fasiglifam (TAK-875; Takeda Pharmaceutical) but its development was terminated during the 3rd phase of clinical study due to hepatotoxicity caused by off-target inhibition of bile acid transporters[2]. Ever since the greatest endeavour in the field has been focused on safety i.e. selectivity and favourable pharmacokinetics[3]. We have developed new, effective and most importantly safe GPR40 agonist as a once-daily drug to manage type II diabetes. Here we show the biological and pharmacological effects of CPL207-280 and fasiglifam as a comparator.

MATERIALS AND METHODS

On-target *in vitro* activity: the compounds' EC50 was determined using hGPR40-CHO cells (Perkin Elmer) with stable expression of hGPR40 receptor and aequorin, whereas insulin secretion assay was performed using MIN6 cells (Addexbio) in KRBH buffer. Insulin level in assay buffer was measured using Mouse Insulin Elisa (Mercodia). Selectivity panel was performed by DiscoverX at the concentration of 10 μM.
Toxicity/Off-targets activity: the compounds' cytotoxicity was determined using Real-Time Glo assay (Promega) in MIN6 and HepG2 (ATCC) cells after 48 h stimulation of the compound in serum free medium.
Off-target screen using Safety47™ Panel and BioMap® assay were performed by DiscoverX at the concentration of 10 μM for Safety47™ and 0.370; 1.1; 3.3; 10 μM for BioMap®.
Determination of compound inhibitory activity against hepatic transportes was performed by Solvo Biotechnology.
Glucose Tolerance Test: *in vivo* effect was examined in 8-10 weeks old male C57BL6 mice using an intraperitoneal glucose tolerance test (IPGTT). Animals were fasted for 12 hours (with free access to water) and at the time point t = 0 received a single dose of the compound administered intravenous (i.v.) and glucose bolus (2 mg/kg) administered i.p. *In vivo* effect in 8-10 weeks old male Wistar Han Rats was examined using IPGTT. Animals were fasted for 12 hours (with free access to water) and at the time point t = 0 received a single dose of the compound administered per os (p.o.) and glucose bolus (2 mg/kg) administered i.p. Animals received also second glucose bolus 6 h after compound administration (at timepoint t = 6h). Glucose lowering effect in 12-14 weeks old male Goto-Kakizaki Rats (GK/MolTac fromTaconic) was examined using IPGTT. Animals were fasted for 6 hours (with free access to water) and at the time point t = -1h (one hour before glucose administration) received a single dose of the compound administered p.o. A single glucose bolus (2 mg/kg) was administered i.p. at the timepoint t = 0. The glucose level in all studies was measured by a standard Accu-Chek®Performa glucometer. Insulin rat serum level after first glucose bolus was measured using Rat Insulin Elisa (Mercodia).
All animal studies were approved by local ethical committee.
Statistical analysis: statistical analysis were done using GraphPad Prism v.5.0. AUC comparison was done using One-Way ANOVA test. Effect in each timepoints between study groups was calculated using Two-Way ANOVA.

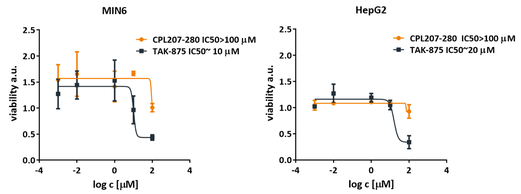
RESULTS

IN VITRO ACTIVITY



RESULTS

IN VITRO TOXICITY



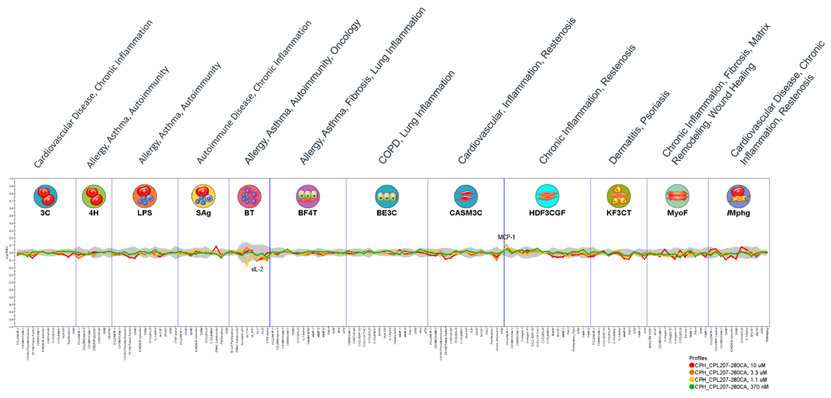
Transporter	IC50 [μM]	
	CPL207-280	TAK-875
MRP2	n.d.	48.1
MRP3	n.d.	31.8
MRP4	131	6.9
BSEP	>100	30
OAT1	245	34.8
OAT2v1	>300	62.8
OATP1A2	118	10.6
OATP1B1	17.9	0.49
OATP1B3	42.9	3.44
NTCP	46.8	53.1

n.d. – no inhibition observed up to 100 μM

OFF-TARGETS' /SELECTIVITY PANEL @10 μM

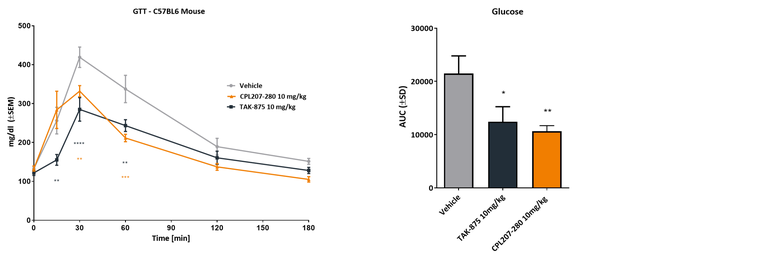
Target	Mode	% response	Target	Mode	% response	Target	Mode	% response	Target	Mode	% response
ADORA2A	Agonist	-19.4	CNR2	Agonist	16.3	OPRK1	Agonist	25.5	AChE	Inhibitor	-2.8
	Antagonist	17.8		Antagonist	-9.4		Antagonist	-1.1	hERG	Blocker	4.6
ADRA1A	Agonist	-3.9	DRD1	Agonist	-0.7	OPRM1	Agonist	20	NAV1.5	Blocker	-7.2
	Antagonist	-8.1		Antagonist	39.2		Antagonist	-7.6	CoX1	Inhibitor	31
ADRA2A	Agonist	24.3	DRD2S	Agonist	17.2	AR	Agonist	-0.6	COX2	Inhibitor	-9.9
	Antagonist	-7.1		Antagonist	-0.9		Antagonist	7.1	MAOA	Inhibitor	1.2
ADRB1	Agonist	-0.1	EDNRA	Agonist	-3.9	GR	Agonist	1.8	PDE3A	Inhibitor	-0.1
	Antagonist	32.3		Antagonist	-8.3		Antagonist	-16.7	PDE4D2	Inhibitor	0.7
ADRB2	Agonist	-1	HRH1	Agonist	0.5	GABAA	Opener	-15.1	INSR	Inhibitor	-20
	Antagonist	45.5		Antagonist	-8.9		Blocker	6.4	LCK	Inhibitor	10.5
AVPR1A	Agonist	-3	HRH2	Agonist	-1.2	HTR3A	Opener	-8.1	ROCK1	Inhibitor	-5
	Antagonist	-17.7		Antagonist	34.2		Blocker	18.3	VEGFR2	Inhibitor	-15
CCKAR	Agonist	-1.9	HTR1A	Agonist	22.1	DAT	Blocker	11.1	FFAR2	Agonist	-1
	Antagonist	-3		Antagonist	-17.9	NET	Blocker	12.5	FFAR3	Agonist	28.5
CHRM1	Agonist	-8.5	HTR1B	Agonist	15.1	SERT	Blocker	12.6	GPR119	Agonist	9.1
	Antagonist	-1		Antagonist	-5.2	CAV1.2	Blocker	-21.1	GPR120	Agonist	0.5
CHRM2	Agonist	32.1	HTR2A	Agonist	-5.4	KvLQT1/minK	Opener	-1.2	GPR55	Agonist	6.3
	Antagonist	-6.3		Antagonist	6.3		Blocker	12.7	GPR142	Agonist	8.5
CHRM3	Agonist	-5	HTR2B	Agonist	-40.8	nAChR9(a4/b2)	Opener	-9.1	GLP1R	Agonist	-2.6
	Antagonist	-3.2		Antagonist	7.2		Blocker	37.4	PPARγ	Agonist	1.2
CNR1	Agonist	63	OPRD1	Agonist	22.4	NMDAR(1A/2B)	Opener	-12.4	PPARα	Agonist	0.9
	Antagonist	-3.9		Antagonist	-9.2		Blocker	-16.4			

BioMAP® ASSAY



RESULTS

IN VIVO ACTIVITY - GLUCOSE TOLERANCE TEST (GTT)



CONCLUSIONS

The biological and pharmacological potency, as well as safety (especially in liver transporter inhibition) of CPL207-280 was higher than that of fasiglifam. CPL207-280 improved glycemic control and showed high activity in insulin secretion in mouse and rats. Therefore we propose CPL207-280 compound as an effective and safe candidate for the T2DM treatment.

REFERENCES

[1] Itoh Y. *et al.* Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. *Nature* (2003) Mar 13;422(6928):173-6
[2] Kaku K. *et al.* Efficacy and safety of fasiglifam (TAK-875), a G protein-coupled receptor 40 agonist, in Japanese patients with type 2 diabetes inadequately controlled by diet and exercise: a randomized, double-blind, placebo-controlled, phase III trial. *Diabetes, Obesity and Metabolism* (2015) 17: 675–681
[3] Wolenski F. *et al.* Fasiglifam (TAK-875) Alters Bile Acid Homeostasis in Rats and Dogs: A Potential Cause of Drug Induced Liver Injury. *Toxicological sciences,* (2017) 157(1), 2017, 50–61

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