Ultra-selective DYRK1A/B Inhibitors Mediated β-Cell Proliferation In Vitro and In Vivo

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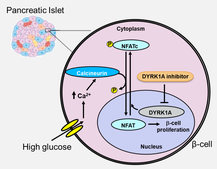
Background

- Impaired β-cell function or β-cell loss is a major unmet need in diabetes and current treatments for diabetes do not address the loss of functional pancreatic β-cells: therefore, there is an urgent need to identify new therapeutic strategies
- DYRK1A, a member of the dual-specificity tyrosine phosphorylation regulated kinase family, is viewed as a promising new target to induce β-cell proliferation via its ability to modulate multiple growth regulatory pathways including NFAT signaling activation and derepression of DREAM complex target genes[1-3]. In addition, the simultaneous inhibition of DYRK1A and DYRK1B has been shown to be more effective than inhibition of DYRK1A alone[4]
- However, there has been a paucity of potent, specific, and orally bioavailable DYRK1A/B inhibitors that did not target the related cdc2-like kinases (CLKs) and glycogen synthase kinase 3β (GSK3β)[5]
- In this study, we present and have characterized two highly potent and selective inhibitors of DYRK1A/B. We utilized these inhibitors to assess the specific role of DYRK1A/B activity in β-cell proliferation



Adapted from Shen et al, Nature, 2015 and Belgardt & Lammert, Diabetes, 2016 [6,7]

Conclusions

- **We have developed multiple small molecule DYRK1A/B inhibitors that are highly specific and potent for DYRK1A and DYRK1B**
- **Biosplice's DYRK1A/B inhibitors promoted β-cell proliferation and increased stimulated insulin secretion in human islets in vitro**
- **Biosplice's DYRK1A/B inhibitors showed high oral bioavailability (>100%) in rodents**
- **Oral administration of SM15238 and SM15268 dose responsively prevented the rise in HbA1c and resulted in sustained increased circulating 4hr fasted insulin levels**
- **Selective DYRK1A/B inhibition promoted β-cell proliferation, increased insulin and islets area without increasing α-cell number and glucagon area in db/db mice**
- **SM15268 45mg/kg reduced body weight gain, dosing regimen optimization will be evaluated**
- **These studies support further evaluation of Biosplice's DYRK1A/B selective inhibitors as a therapeutic option for Diabetes**

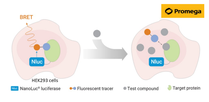
Results

Fig. 1: SM15238 and SM15268 are highly potent and selective DYRK1A/1B inhibitors that induced INS-1 β-cell proliferation in vitro

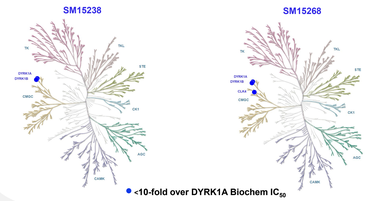
A Biochemical and Target Engagement profiles

Compound	Biochemical IC_{50} (μM)		Target Engagement IC_{50} (μM)							% Full kinome with ≥50% inhibition @ 1 μM
	DYRK1A	GSK3β	DYRK1A	DYRK1B	DYRK2	CLK1	CLK2	CLK3	CLK4	
Harmine	0.036	9.992	0.512	0.650	5.286	0.917	3.685	9.980	0.762	NA
SM15238	0.001	1.998	0.017	0.035	0.961	0.629	1.169	5.717	0.533	2.0
SM15268	0.001	9.928	0.022	0.040	1.354	0.493	0.823	5.665	0.285	1.8

Biochem
IC_{50} <0.005
0.01≤IC_{50}<0.05
IC_{50} >1
Target Engagement
IC_{50} <0.03
0.03≤IC_{50} <0.1
0.02<IC_{50}<0.4
IC_{50} >0.4



B Full kinome profiles



C In vitro INS-1 β-cell proliferation

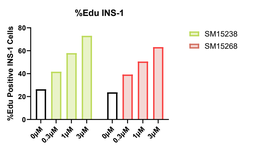
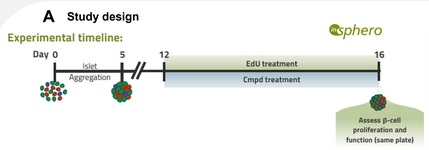
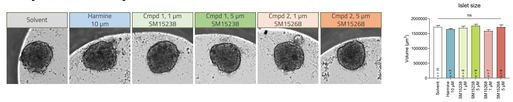


Fig. 2: SM15238 and SM15268 induced β-cell proliferation and increased insulin secretion in human islets in vitro

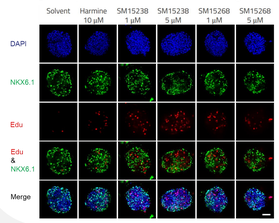
A Study design



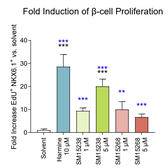
B Compound treatment did not affect human islet microtissue morphology or result in any obvious toxicity



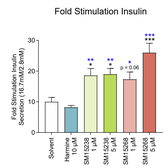
C Representative 3D confocal images



D Compound treatment increased human β-cell proliferation



E Compound treatment increased stimulated insulin secretion



Mean + SEM; *p<0.05, ** p<0.01 *** p<0.001 reported from ANOVA vs. Solvent Control using Dunnett's correction (black asterisk) or unpaired t-test vs. Solvent control (blue asterisk)

Fig. 3: Study design to evaluate the effects of SM15238 and SM15268 in db/db mouse model and effects of treatments on body weight

A Study design in male db/db mouse efficacy model

Treatment	Dose (mg/kg)	Frequency	Route	n/group
Vehicle	NA	QD	PO	8
SM15238	15	QD	PO	8
SM15238	45	QD	PO	8
SM15268	15	QD	PO	8
SM15268	45	QD	PO	8
db/+ Vehicle	NA	QD	PO	8

Weekly basis: d0, d7, d14, d21, d28, d35
- BW
- Non-fasted glucose
- Fasted glucose
- Fasted Insulin
- Glucagon

B Body Weight

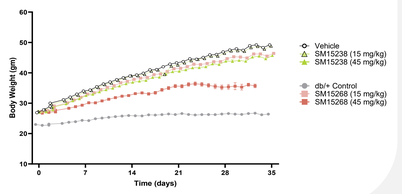
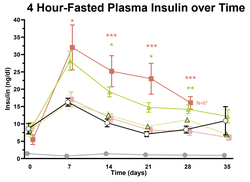


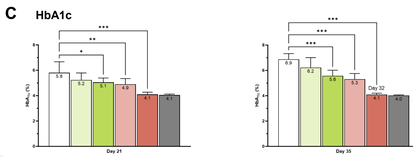
Fig. 4: DYRK1A/B inhibition reduced circulating glucose, increased plasma insulin levels and reduced hemoglobin A1c in db/db mouse model

A 4 Hour-Fasted Glucose over Time

B 4 Hour-Fasted Plasma Insulin over Time

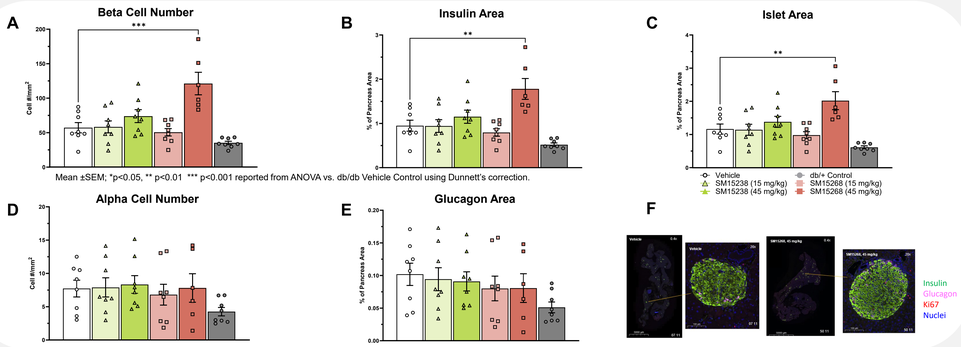


C HbA1c



Lower Detection Limit of the Assay = 4.0%
*p<0.05, ** p<0.01 *** p<0.001 reported from ANOVA vs. db/db Vehicle Control using Dunnett's correction
‡One out of eight SM15268 45 mg/kg mice was euthanized on day 18 due to lower food intake and reduced body weight gain. Another one was found dead on day 26. Cause of death unknown

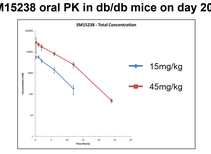
Fig. 5: DYRK1A/B inhibition promoted β-cell proliferation, increased insulin and islets area without increasing α-cell number and glucagon area in db/db mouse model



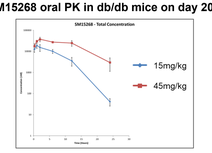
Mean ±SEM; *p<0.05, ** p<0.01 *** p<0.001 reported from ANOVA vs. db/db Vehicle Control using Dunnett's correction.

Fig. 6: SM15238 and SM15268 showed high oral bioavailability

A SM15238 oral PK in db/db mice on day 20



B SM15268 oral PK in db/db mice on day 20



C SM15238 and SM15268 plasma exposures in CD1 mice (10 mg/kg) and in db/db mice (15 and 45 mg/kg at day 20) when administered orally

Compound	Dose (mg/kg)	AUC (ng.h/mL)	C_{max} (ng/mL)	%F	Unbound C_{avg}/0.3μM*
SM15238	10	10652	2837	132	NA
	15	10269	2286	NA	0.2
	45	55476	10859	NA	1.2
SM15268	10	22691	5297	137	NA
	15	58898	7731	NA	0.8
	45	206710	15259	NA	2.8

*the lowest concentration tested in in-vitro INS-1 β-cell proliferation assay

Figure Legends and Methodology

- **Fig. 1**: (**A**) Table listing biochemical DYRK1A and GSK3β kinase inhibition IC_{50} and the target engagement of CLK/DYRK family members by SM15238 and SM15268. Biochemical kinase assay IC_{50} values were determined using the Thermo Fisher Scientific Z-LYTE™ platform. Target engagement assay IC_{50} values were determined using the Promega NanoBRET TE Intracellular Kinase Assay platform in transiently transfected HEK293T cells. IC_{50} values were determined from 10-point dose response curves using non-linear regression curve fit. Full kinome data is from Thermo Fisher Scientific SelectScreen™ Profiling Service using compounds at 1μM (**B**) Dendrogram of the human kinome. Kinases inhibited at 90% or more by Biosplice compounds and showing an IC_{50} less than 10-fold over DYRK1A IC_{50} are highlighted with a blue circle and labeled accordingly. (**C**) In vitro β-cell proliferation assay using rat INS-1 cells treated for 2 days with SM15238 or SM15268 at the indicated concentrations.
- **Fig. 2**: (**A**) InSphero study outline using standardized islet model, 3D InSight™ human Islet Microtissues (hIsMTs) developed by InSphero. hIsMTs were produced by optimized dissociation and controlled scaffold free reaggregation of primary islet cells and were treated for 4 days with each of the assessed compounds at the given concentrations using a Tecan D300e digital dispenser. Culture medium was exchanged and hIsMTs were dosed for each compound every 2 or 3 days. (**B**) Representative brightfield images of hIsMTs treated with compounds for 4 days. Islet size, measured by 3D confocal microscopy. Data represent the mean + SEM of 1 donor with n = 6 – 10 technical replicates, as indicated on the graph. (**C**) Representative 3D confocal microscopy images of human islets treated with solvent (DMSO), Harmine as positive control or Biosplice compounds and Edu for 4 days, fixed and stained for DAPI/NKX6.1/EdU. All images were generated with the same thresholding settings. Scale bar: 50 μm. (**D**) 3D image analysis of human islets microtissues treated for 4 days showing fold change of proliferating β-cell count (Edu$^+$NKX6.1$^+$) induced by compounds vs. solvent (DMSO). (**E**) Functional analysis of hIsMTs treated with compounds for 4 days. For the GSIS assay, the hIsMTs were washed once with Krebs Ringer HEPES buffer (KRHB) containing 2.8 mM Glucose, then equilibrated in KRHB containing 2.8 mM Glucose for 1 hour. GSIS was performed sequentially in KRHB containing 2.8 mM and 16.7 mM Glucose for 2 hours each. Secreted insulin was quantified using ALPCO Stellux® Chemi Human Insulin ELISA.
- **Fig. 3**: (**A**) Schematic of the study design to assess the effects of SM15238 and SM15268 in ~5-week-old male db/db mouse model. (**B**) Effects of treatments on body weights.
- **Fig. 4**: (**A**) Effects of treatments on 4-hour fasted glucose levels measured every week from blood collected via tail vein using Roche Aviva glucometer. (**B**) Effects of treatments on 4-hour fasted insulin levels measured every week with Elisa using blood samples collected via tail vein into EDTA coated K2E tubes. (**C**) Effects of treatments on HbA_{1c} at Day 21 and Day 35 of the study using blood samples collected via tail vein into EDTA coated K2E tubes.
- **Fig. 5**: Histology analysis on pancreas collected at the end of study showing the treatment effects on i) β-cell count (**A**) and insulin area (**B**) identified by insulin staining, ii) on islets area (**C**) and iii) on α-cell count (**D**) and glucagon area (**E**) identified by glucagon staining. (**F**) Representative images of pancreatic islets histology analysis. Visiopharm software was used for image analysis.
- **Fig. 6**: Plasma PK profile of SM15238 (**A**) and SM15268 (**B**) measured at Day 20 of the study. (**C**) Table highlighting the C_{max} and AUC of both molecules plotted in panels A and B. Compounds were administered daily and PO.

References

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