A Dual Inhibitor of DYRK1A and GSK3β for β-Cell Proliferation: Aminopyrazine Derivative GNF4877

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Loss of β-cell mass and function can lead to insufficient insulin levels and ultimately to hyperglycemia and diabetes mellitus. The mainstream treatment approach involves regulation of insulin levels; however, approaches intended to increase β-cell mass are less developed. Promoting β-cell proliferation with low-molecular-weight inhibitors of dual-specificity tyrosine-regulated kinase 1A (DYRK1A) offers the potential to treat diabetes with oral therapies by restoring β-cell mass, insulin content and glycemic control. GNF4877, a potent dual inhibitor of DYRK1A and glycogen synthase kinase 3β (GSK3β) was previously reported to induce primary human β-cell proliferation *in vitro* and *in vivo*. Herein, we describe the lead optimization that lead to the identification of GNF4877 from an aminopyrazine hit identified in a phenotypic high-throughput screening campaign measuring β-cell proliferation.

Introduction

More than 400 million people worldwide are living with diabetes which results from insufficient mass and/or impaired function of pancreatic insulin-producing β-cells.[1–3] Progress towards the pharmacological driven expansion of functional β-cells faces multiple challenges, such as limited understanding of the signaling pathways relevant for human β-cell growth and the extremely low levels of proliferation capacity of adult human β-cells, even after mutagenic stimulation.[4] Despite these challenges, there has been steady progress identifying molecules that could potentially increase proliferation of human β-cells.[4] For example, circulating factors derived from hepatocytes were shown to promote proliferation of β-cells.[5] SerpinB1, a conserved protease inhibitor, enhanced β-cell proliferation in zebrafish, mice and humans.[6] Diarylamide compound WS6 induced human β-cell proliferation in a dispersed islet proliferation assay and in intact human islet cultures.[7] Harmine and its derivatives were reported to induce human β-cell proliferation both *in vitro* and in transplanted human islets in NOD-SCID mice. It was elucidated that harmine's inhibition of dual-specificity tyrosine-regulated kinase 1A (DYRK1A) contributes to β-cell proliferation through the attenuation of nuclear factor phosphorylation in activated T cells (NFAT).[8–12] Besides harmine, numerous DYRK1A inhibitor scaffolds have been reported (Figure 1);[13–34] including 5-iodotubercidin (5-IT),[16] INDY,[17] and selective DYRK1A inhibitor GNF2133,[18] which have shown to promote human β-cell proliferation.

β-Cell proliferation could further be enhanced by combining DYRK1A inhibitors with different mechanisms of action. Stewart and co-workers recently demonstrated that the combination of a glucagon-like peptide-1 receptor (GLP-1R) agonist with a DYRK1A inhibitor could induce a synergistic increase in replication and numbers of human β-cells.[35] In Shen et al., we reported aminopyrazine derivative **1** (GNF4877, Figure 1), a dual inhibitor of DYRK1A and GSK3β. This compound showed that inhibition of both DYRK1A and glycogen synthase kinase 3β (GSK3β) can have a synergistic effect enhancing β-cell proliferation, compared with DYRK1A inhibition alone.[36] We found that human islets treated with **1** retain functionality both *in vitro* and *in vivo* after transplantation into diabetic mice.[36] Oral dosing of **1** in diabetic mice induced β-cell proliferation, increased β-cell mass and insulin content and improved glycemic control.[36] In this report, we describe the lead optimization efforts which led to the discovery of **1**.

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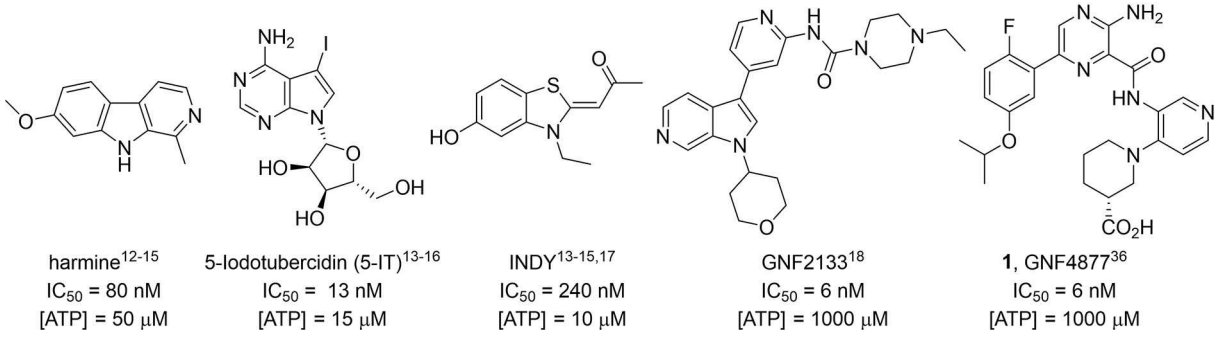


Figure 1. Selected small-molecule DYRK1A inhibitors (reported literature IC_{50}).[12–18,36]

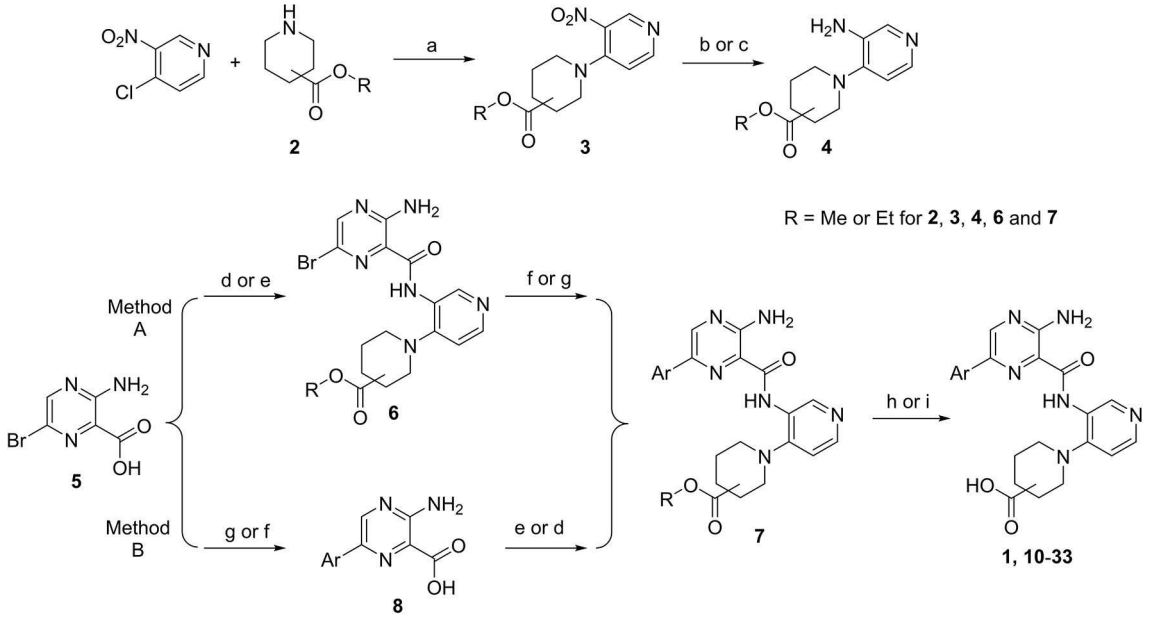
Results and Discussion

Chemistry

The aminopyrazine analogues in this report, except for compound **9** (Table 1) whose synthesis was previously described,[37] were synthesized through the general reaction sequence outlined in Scheme 1. The synthesis began with an aromatic nucleophilic substitution reaction between 4-chloro-3-nitropyridine and a cyclohexylamine compound **2** with R being either methyl or ethyl to give the nitropyridine **3**, which was subsequently reduced to the aminopyridine building block **4** by catalytic hydrogenation. The synthesis of intermediate **7** was achieved by permutating between amide and Suzuki coupling conditions with 3-amino-6-bromopyrazine-2-carboxylic acid (**5**) and aminopyridine **4**. By using method A, amide bond formation between **4** and **5** afforded compound **6**, which was then subjected to Suzuki coupling conditions with the corresponding boronic acid (or boronic ester) to yield **7**. Alternatively, using method B, Suzuki coupling of aryl bromide **5** with the corresponding boronic acid (or boronic ester) afforded compound **8**, which was subjected to amide bond formation with aminopyridine **4** to yield **7**. Hydrolysis of methyl or ethyl ester afforded the final aminopyrazine products (**1**, **10**–**33**).

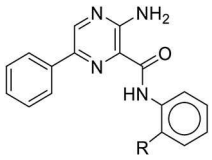
Lead optimization

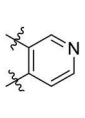
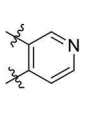
A safe orally administered small-molecule drug that can selectively expand functional β-cell mass would be a highly desirable option for diabetic patients over routine insulin injections. The aim of the medicinal chemistry effort described here was to discover potent orally bioavailable small-molecule DYRK1A and GSK3β dual inhibitors that can proliferate human



Scheme 1. Synthesis of the aminopyrazine analogues. a) K_2CO_3, DMF, 25 °C, 4 h; b) Pd/C (10%), H_2 (balloon), MeOH, 25 °C, 16 h; c) Pd/C (10%), H_2 (50 psi), EtOAc, 25 °C, 3 h; d) **4**, HATU, DMF, 25 °C, 2 h; e) **4**, EDC HCl, HOAt, DMF, 25 °C, 18 h; f) $ArB(OH)_2$ or ArBpin, $Pd(dppf)Cl_2$, Et_3N, CH_3CN, 90 °C, 4 h; g) $ArB(OH)_2$ or ArBpin, $Pd(PPh_3)_4$, Na_2CO_3, dioxane/H_2O, 90 °C, 3 h; h) LiOH (aqueous, 1.0 M), EtOH, 25 °C, 16 h; i) LiOH (aqueous, 1.0 M), THF/MeOH, 25 °C, 4–16 h.

Table 1. Structure-activity relationship (SAR) of aminopyrazine derivatives on enzymatic DYRK1A and GSK3β inhibition.



Compound	R	DYRK1A IC_{50}[a] [μM]	GSK3β IC_{50}[a] [μM]
9		0.039	0.003
10		0.100	0.040
11		0.113	0.088
12		4.51	3.747
13		22.68	0.288
14		>50	0.280
15		25.02	25.83
16		>50	1.478
17		0.583	0.085
18		0.427	0.019
19		0.068	0.006
20		0.606	0.088
21		2.426	0.039
22		2.493	0.033

[a] Obtained from three or more independent experiments. ATP concentration = 1.0 mM.

β-cells. Our work started from the aminopyrazine derivative **9**, which was identified from a 850,000 compound high-throughput screening (HTS) campaign measuring β-cell proliferation by using growth-arrested reversibly immortalized mouse β-cell line R7T1 (EC_{50} = 0.018 μM).[7] Compound **9** was later demonstrated to be a dual inhibitor of DYRK1A and GSK3β.[36] Further profiling however showed that compound **9** was unable to proliferate primary rat β-cells. In contrast, the carboxylic acid **10**, albeit less potent in the R7T1 assay (EC_{50} = 0.33 μM), showed moderate proliferative activity in rat islet β-cell proliferation assay (EC_{50} = 3.5 μM). We conjectured that the lack of primary islet activity observed in **9** could be attributed to its basic amino group and a related propensity to sink in the lysosome.[38] Therefore, further optimization was focused on carboxylic acids. The enzymatic DYRK1A activity was used as the primary driver for lead optimization, while retaining activity on both DYRK1A and GSK3β as basic criteria for selecting compounds for further profiling in a rat islet proliferation assay.

Docking of **10** into the ATP binding pocket of DYRK1A using an in-house co-crystal structure[18] showed that the pyrazine core forms two hydrogen bonds in the hinge area, one between the pyrazine nitrogen and backbone NH of L241 (hidden from view), and the other between the pyrazinylamino group and the backbone carbonyl oxygen of E239 (Figure 2). Additionally, **10** forms H-bonds with the side chain of K188 via the pyridine nitrogen and with N244 via the carboxyl group. The interaction with K188 proved to be critical, as moving (**11**–**13**) or removing (**14**) the pyridyl nitrogen resulted in partial or complete loss of enzymatic DYRK1A inhibitory activity (Table 1).

We further evaluated the structure-activity relationship (SAR) by replacing the piperidine-4-carboxylic acid ring (Table 1). The ring size of the cyclic amine had a significant impact on the activity on DYRK1A, where four- and five-membered rings decreased activity substantially (**15**, **16** vs **10**). Introducing a fluorine atom at the α position relative to the carboxylic acid caused a sixfold reduction in potency (**17** vs **10**). Moving the carboxylic acid from the 4-position to the 3-position of the piperidine ring maintained the activity, but only when the stereogenic center is in the *R* configuration (**19** vs **10**), whereas the *S* enantiomer (**18**) was less potent. Replacing the piperidine ring in **19** with a piperazine ring caused a ninefold decrease in inhibition (**20** vs **19**). Homologating the carboxylic acid by one carbon at the 3-position of the piperidine ring resulted in more than a 35-fold decrease in inhibition independently of stereochemistry (**21** and **22** vs **19**).

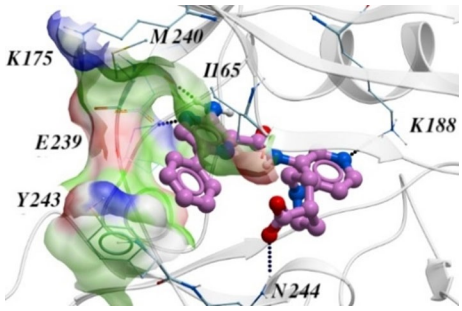
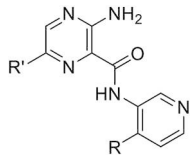


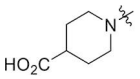
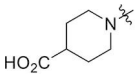
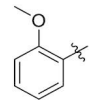
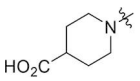
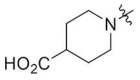
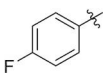
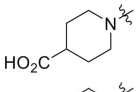
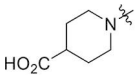
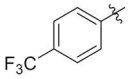
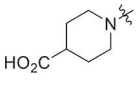
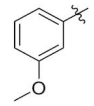
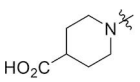
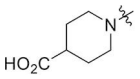
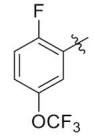
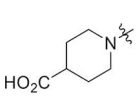
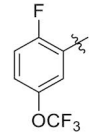
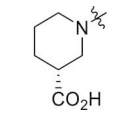
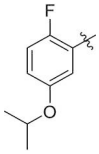
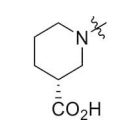
Figure 2. Molecular docking model of **10** in the DYRK1A crystal structure (PDB ID: 6UIP).

To potentially fill the unoccupied space surrounding the phenyl ring at the 6-position of the pyrazine core (Figure 2), we set out to explore whether substituted aromatic groups (designated R′) could increase potency on DYRK1A (Table 2). Introduction of methyl and fluoro substituents at the *ortho* and *para* position resulted in compounds two to three times less potent than the unsubstituted analogue (**23**, **24**, **27**, **28** vs **10**) whereas other slightly larger substituents led to about a tenfold loss in activity (**25**, **26**, **29** vs **10**). Modeling studies suggested that larger substituents in the *ortho* position bring the phenyl out of plane and this possibly accounts for the difference in activity between *ortho*-substituted compounds (**23**, **24** vs **26**). The lower potency of *para*-substituted compounds with larger groups (**29** vs **27**, **28**) can be rationalized by a possible clash with Y243. Interestingly, substituents at the *meta* position yielded compounds with improved potency (**30**, **31** vs **10**), as they likely avoided twisting of the ring out of plane and prevented negative interactions with Y243. Further inhibitory potency improvement was observed when combining a small substitution (F) at the *ortho* position and a larger substitution (trifluoromethoxy) at the *meta* position onto the phenyl ring especially when combined with the (*R*)-piperidine-3-carboxylic acid at R (**32**, **33**). Finally, replacement of the *meta*-trifluoromethoxy substitution with an isopropoxy group led to compound (**1**) with single-digit nanomolar potency. Docking of **1** into the DYRK1A crystal structure revealed that the isopropoxy substituent fits into the hydrophobic pocket created by residues K175, I165, M240, and Y243, which may explain the increased inhibitory potency (Figure 3).

Compared with **10**, aminopyrazine **1** demonstrated a 14-fold improvement in activity for DYRK1A and 2.5-fold for GSK3β and was selected for further profiling *in vitro* and *in vivo*. Consistent with the increased biochemical potency on both DYRK1A and GSK3β, compound **1** showed 23-fold increase in potency in the rat islet β-cell proliferation assay compared to compound **10** (Table 3). Importantly, **1** also demonstrated good proliferative activity in the human islet β-cell assay. Compound **1** showed acceptable physicochemical and *in vitro* ADME properties, with desirable PSA ($\leq$140) and clog*P* ($<$5), good permeability, moderate metabolic stability and no major CYP inhibition or hERG liabilities. Solubility of **1**, however, was low, as measured by high throughput thermodynamic solubility. On a broad kinase panel, compound **1** showed comparable inhibition for SYK and modest inhibitions on ROS, CDK2 and Axl, as previously reported.[36] In addition, **1** has preferential inhibitory activity for both DYRK1A and DYRK1B subtypes, compared to DYRK2.[39]

Table 2. The effects of phenyl ring substitution on enzymatic DYRK1A and GSK3β inhibition.



Compound	R′	R	DYRK1A IC_{50}[a] [μM]	GSK3β IC_{50}[a] [μM]
23			0.243	0.093
24			0.330	0.06
25			1.071	0.469
26			1.120	0.063
27			0.187	0.041
28			0.281	0.067
29			1.023	0.08
30			0.074	0.147
31			0.097	0.106
32			0.075	0.800
33			0.055	0.085
1			0.006	0.016

[a] Obtained from three or more independent experiments. ATP concentration = 1.0 mM

In vivo studies

In *in vivo* pharmacokinetic (PK) studies in mice dosed intravenously (IV), compound **1** displayed moderate to high clearance (CL) at approximately 74% of mouse hepatic blood flow, moderate volume of distribution (*V*ss) and short half-life ($t_{1/2}$; Table 4). Following a single 10 mg/kg oral (PO) administration, **1** showed low to moderate oral bioavailability (*F*%). Based on single dose oral PK data, the regimen of 50 mg/kg twice daily oral dosing in 0.5% *w/v* MC/0.5% *w/v* Tween 80 suspension was chosen for testing in a diabetes efficacy study

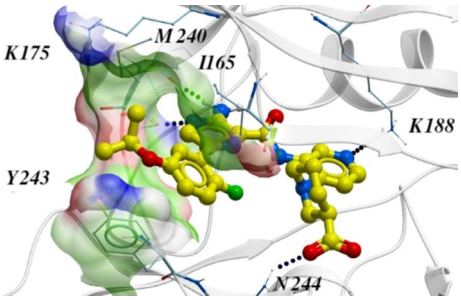


Figure 3. Molecular docking model of **1** in the DYRK1A crystal structure (PDB ID: 6UIP).

Table 3. Enzymatic and primary islet activity, ADME and safety profiling for compounds **10** and **1**.

Compound	**10**	**1**
Primary islet activity		
rat islet EC_{50}[a] [μM] (% efficacy[b])	3.5 (100)	0.15 (180)
human islet EC_{50}[a] [μM] (% efficacy[b])	0.76 (100)	0.54 (430)
Cardiac ion channels		
binding P4 [μM][18]	>30	n.d.[d]
QPatch S8 [μM][18]	>30	n.d.[d]
patch clamp Nav1.5 [μM][18]	n.d.[d]	>50
ADME		
Microsomal stability (% remaining)[c]		
human	43.0	39.0
rat	69.0	33.3
mouse	61.0	20.64
CYP450 inhibition [μM]		
CYP2C9	>25	8.6
CYP2D6	>25	20.3
CYP3A4 I	>25	>25
CYP3A4 II	>25	>25
thermodynamic solubility [μM]	<2	14.1
logPe PAMPA (pH 6.8)	−5.4	−4.7
mouse plasma protein binding	n.d.[d]	99.5%

[a] Obtained from three or more independent experiments. [b] Proliferation % efficacy relative to the assay standard **10** set at 100%. [c] After 30 min of incubation. Microsomes derived from pieces of endoplasmic reticulum (ER) were used. [d] Not determined.

Table 4. Pharmacokinetic (PK) parameters for **1** in CD-1 mice.

	(IV) [a]	(PO) [b]
dose [mg kg^{-1}]	2	10
CL [mL min^{-1} kg^{-1}]	66.8	
Vss [L kg^{-1}]	1.85	
AUC [h nM]	1013	805
C_{max} [nM]	2819	269
t_{max} [h]	0.03	1.0
$t_{1/2}$ [h]	0.91	2.32
F%		15.9

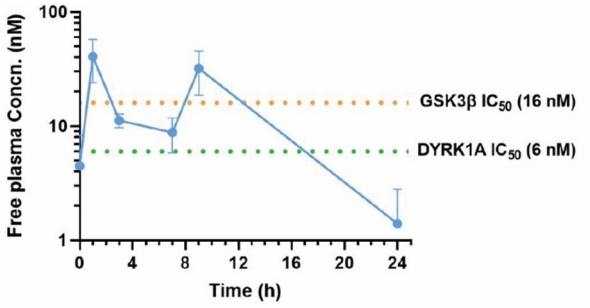
[a] Solution formulation (2.5 mg/mL in 50 mM Na_2CO_3 buffer, pH 9.3). [b] Suspension formulation (1.0 mg/mL in 0.5% *w/v* MC/0.1% *w/v* Tween 80).

(rat insulin promoter and diphtheria toxin A (RIP-DTA) mice). Compound **1**-treated diabetic mice demonstrated a significant increase in β-cell mass and improved glycemic control.[36] The plasma exposure of **1** was determined on day 10. The exposure was significantly higher in diabetic mice than predicted from single dose PK study in normal CD-1 mice. The free concentrations of **1** in the efficacy study exceeded DYRK1A enzymatic IC_{50} (6 nM) and was transiently above the GSK3β enzymatic IC_{50} (16 nM) for the dosing interval, as shown in Figure 4.

As reported by Shen et al., human islets treated with **1** *in vitro* demonstrated increases in DNA and ATP content, and an increase in islet equivalent units compared with vehicle-treated cultures.[36] In addition, human islets treated with **1** maintained insulin secretion capacity. Compound **1** stimulated proliferation of functional primary human β-cells *in vivo* when a suboptimal dose of human islets were transplanted under the kidney capsule of immune-compromised streptozotocin (STZ)-treated mice.[36] In a separate *in vivo* study using RIP-DTA mice, **1** demonstrated a significant increase in β-cell mass and improved glycemic control.[36]

Conclusions

In conclusion, the aminopyrazine scaffold was identified from a phenotypic high-throughput screening campaign measuring β-cell proliferation using mouse R7T1 β-cells.[7] Lead optimization efforts starting from screening hit **9** led to the identification of a potent dual DYRK1A and GSK3β inhibitor aminopyrazine **1** (GNF4877). SAR studies on the aminopyrazine scaffold were driven by an enzymatic DYRK1A inhibition assay using a structure-guided approach. The DYRK1A and GSK3β dual inhibitor GNF4877 showed low-nM inhibition on both targets and demonstrated unambiguous β-cell proliferation *in vitro* and *in vivo*. As there is the possibility that long-term inhibition of GSK3β might cause adverse effects,[18,40] GNF4877 was not progressed beyond preclinical research. However, the work on the aminopyrazine series laid a foundation for discovering selective DYRK1A inhibitors to expand functional human β-cell mass.[18,36]



Total plasma exposure		Free drug exposure	
C_{max} [nM]	AUC_{0-24} [h nM]	C_{max} [nM]	AUC_{0-24} [h nM]
9383	81130	47	406

Figure 4. Plasma exposure of **1** in RIP-DTA mice following 50 mg/kg twice daily oral dosing in 0.5% *w/v* MC/0.5% *w/v* Tween 80 suspension formulation at day 10. Mean ± SD; $n = 3$. Mouse plasma protein binding of **1** is 99.5%.

Experimental Section

Biology

All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Genomics Institute of the Novartis Research Foundation (GNF) and strictly followed the NIH guidelines for the humane treatment of animals. R7T1 β-cells were obtained from Dr. S. Efrat (Tel Aviv University) and expanded in growth medium.[36] Human islets were obtained from Prodo Laboratories, Inc. (Irvine, CA, USA), University of Minnesota and University of Miami, in accordance with internal review board (IRB) ethical guidelines for the use of human tissue.[36] Informed consent was obtained from the donors for studies using human islets.

DYRK1A and GSK3β biochemical assays were used as previously discribed.[18,36] A β-cell proliferation assay with growth-arrested reversibly immortalized mouse β-cell line R7T1 was used as previously discribed.[7,18,36] Rat and human primary β-cell proliferation assays were used as previously discribed.[7,18,36]

Chemistry

All commercial reagents and solvents were used without purification. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 or 500 MHz spectrometer using solvent residual peak as the internal standard. TLC analyses were carried out on aluminum sheets precoated with silica gel 60 F254, and UV radiation was used for detection. Flash column chromatography was performed on Teledyne ISCO CombiFlash systems. The purity of the final compounds was determined to be >95% by ^{1}H NMR, LCMS and/or CHN elemental analysis. LRMS (ESI) data were recorded using an Agilent 6520 Accurate-Mass Q-TOF LC–MS system with HPLC-Chip Cube interface and an Agilent 1200 HPLC. Elemental analysis was performed at Midwest Microlab (Indianapolis, IN 46250).

Syntheses

*Methyl 1-(3-nitropyridin-4-yl)piperidine-4-carboxylate (**3 a**)*: A suspension of 4-chloro-3-nitropyridine (**1**; 3.17 g, 20.0 mmol), methyl piperidine-4-carboxylate (**2 a**; 2.86 g, 20.0 mmol) and K_2CO_3 (5.53 g, 40.0 mmol) in MeOH (100 mL) was stirred at 25 °C for 4 h, and then concentrated under reduced pressure, mixed with water (200 mL), and extracted with CH_2Cl_2 (3×40 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to column chromatography (hexanes/EtOAc 4:1 to 1:99) to afford **3 a** (4.40 g, 83%). ^{1}H NMR ([D$_6$]DMSO, 500 MHz): δ = 8.75 (s, 1H), 8.34 (d, J = 6.1 Hz, 1 H), 7.18 (d, J = 6.1 Hz, 1 H), 3.63 (s, 3H), 3.36–3.40 (m, 2H), 3.05–3.10 (m, 2H), 2.62–2.70 (m, 1H), 1.88–1.95 (m, 2H), 1.62–1.70 (m, 2H). LRMS (ESI) m/z 266.1 $[M+H]^+$.

*Methyl 1-(3-aminopyridin-4-yl)piperidine-4-carboxylate (**4 a**)*: To a solution of methyl 1-(3-nitropyridin-4-yl)piperidine-4-carboxylate (**3 a**; 4.24 g, 16.0 mmol) in MeOH (100 mL) was added Pd/C (10 wt. % loading, 424 mg). The resultant mixture was stirred under H_2 (balloon) for 16 h, and filtered through Celite. The filtrate was evaporated to give **4 a** (3.43 g, 91%). ^{1}H NMR ([D$_6$]DMSO, 500 MHz): δ = 7.90 (s, 1H), 7.72 (d, J = 5.2 Hz, 1 H), 6.75 (d, J = 5.2 Hz, 1 H), 4.73 (br s, 2H, NH), 3.63 (s, 3H), 3.20–3.23 (m, 2H), 2.54–2.60 (m, 2H), 2.42–2.52 (m, 1H), 1.89–1.94 (m, 2H), 1.75–1.83 (m, 2H). LRMS (ESI) m/z 236.3 $[M+H]^+$.

*Methyl 1-(3-(3-amino-6-bromopyrazine-2-carboxamido)pyridin-4-yl)piperidine-4-carboxylate (**6 a**)*: To a suspension of 3-amino-6-bromopyrazine-2-carboxylic acid (**5**; 2.18 g, 10.0 mmol) and methyl 1-(3-aminopyridin-4-yl)piperidine-4-carboxylate (**4 a**; 2.35 g, 10.0 mmol) in DMF (50 mL) was added HATU (4.56 g, 12.0 mmol) and DIEA (5.23 mL, 30.0 mmol) to result in a mixture that was stirred at 25 °C for 2 h, poured into saturated Na_2CO_3 solution (400 mL) and extracted with EtOAc (3×120 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated. The residue was subjected to ISCO chromatography (0–5% MeOH/CH_2Cl_2 eluent) to give **6 a** (3.13 g, 72%). ^{1}H NMR ([D$_6$]DMSO, 500 MHz): δ = 10.05 (s, 1H, NH), 9.34 (s, 1H), 8.47 (s, 1H), 8.27 (d, J = 5.3 Hz, 1 H), 7.80 (br s, 2H, NH_2), 7.17 (d, J = 5.3 Hz, 1H), 3.64 (s, 3H), 3.13–3.18 (m, 2H), 2.76–2.81 (m, 2H), 2.53–2.61 (m, 1H), 1.92–2.03 (m, 4H). LRMS (ESI) m/z 435.3 $[M+H]^+$.

*Methyl 1-(3-(3-amino-6-phenylpyrazine-2-carboxamido)pyridin-4-yl)piperidine-4-carboxylate (**7 a**)*: A mixture of methyl 1-(3-(3-amino-6-bromopyrazine-2-carboxamido)pyridin-4-yl)piperidine-4-carboxylate (**6 a**; 870.6 mg, 2.0 mmol), phenylboronic acid (292.6 mg, 2.4 mmol), $Pd(PPh_3)_4$ (115.8 mg, 0.10 mmol) and Na_2CO_3 (635.9 mg, 6.0 mmol) was heated at 90 °C for 3 h, poured into water (200 mL), and extracted with CH_2Cl_2 (3×40 mL). The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated to result in a residue which was subjected to chromatography (0–5% MeOH/CH_2Cl_2) to give **7 a** (66.2 mg, 77%). ^{1}H NMR ([D$_6$]DMSO, 500 MHz): δ = 10.38 (s, 1H, NH), 9.37 (s, 1H), 8.97 (s, 1H), 8.29 (d, J = 5.3 Hz, 1H), 8.06–8.11 (m, 2H), 7.79 (br s, 2H, NH_2), 7.38–7.47 (m, 3H), 7.21 (d, J = 5.3 Hz, 1H), 3.50 (s, 3H), 3.14–3.22 (m, 2H), 2.70–2.80 (m, 2H), 2.44–2.49 (m, 1H), 1.92–2.00 (m, 2H), 1.70–1.83 (m, 2H); ^{13}C NMR ([D$_6$]DMSO, 125 MHz): δ = 173.87, 163.63, 154.39, 149.74, 145.94, 145.42, 141.23, 138.44, 135.38, 128.77, 128.29, 125.17, 122.89, 114.92, 51.40, 50.34, 39.31, 27.95. LRMS (ESI) m/z 433.2 $[M+H]^+$.

*1-(3-(3-Amino-6-phenylpyrazine-2-carboxamido)pyridin-4-yl)piperidine-4-carboxylic acid (**10**)*: To a suspension of methyl 1-(3-(3-amino-6-phenylpyrazine-2-carboxamido)pyridin-4-yl)piperidine-4-carboxylate (**7 a**; 432.5 mg, 1.0 mmol) in a mixed solvent of THF and MeOH (2.5:1, v/v, 18 mL) was added LiOH (1.0 M, 2.0 mL, 2.0 mmol) to result in a mixture which was stirred at 25 °C for 16 h, and then concentrated under reduced pressure. The residue was dissolved into water (100 mL), washed with CH_2Cl_2 (15 mL), and then acidified with an aqueous HCl solution (1.0 M, 2.0 mL, 2.0 mmol). The solid precipitate was collected by filtration, and dried under reduced pressure to afford **10** (410 mg, 98%). ^{1}H NMR ([D$_6$]DMSO, 500 MHz): δ = 10.46 (s, 1H), 8.98 (s, 1H), 8.68 (s, 1H), 8.34 (d, J = 6.9 Hz, 1H), 8.18–8.21 (m, 2H), 7.67 (br s, 2H, NH_2), 7.46–7.51 (m, 2H), 7.38–7.43 (m, 2H), 3.94–4.02 (m, 2H), 3.19–3.27 (m, 2H), 2.51–2.59 (m, 1H), 1.86–1.94 (m, 2H), 1.63–1.74 (m, 2H); ^{13}C NMR ([D$_6$]DMSO, 125 MHz): δ = 175.23, 165.00, 156.34, 154.37, 145.40, 138.90, 138.69, 137.90, 135.60, 128.80, 128.42, 125.57, 123.10, 122.80, 113.24, 48.24, 39.26, 27.71. LRMS (ESI) m/z 419.2 $[M+H]^+$. Anal. calcd. (%) for $C_{22}H_{22}N_6O_3$: C 63.15, H 5.30, N 20.08; found: C 62.83, H 5.28, N 19.97; purity ≥99.6%.

*(R)-Ethyl 1-(3-nitropyridin-4-yl)piperidine-3-carboxylate (**3 b**)*: To a suspension of 4-chloro-3-nitropyridine (12.68 g, 80.0 mmol) in ethanol (100 mL) were added K_2CO_3 (11.06 g, 80.0 mmol.) and a solution of (*R*)-ethyl piperidine-3-carboxylate (**2 b**; 12.58 g, 20.0 mmol) in ethanol (20 mL) at 0 °C. The resultant mixture was stirred at 0 °C for 4.5 h, poured into water (500 mL), and extracted with CH_2Cl_2 (3×100 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to column chromatography (20–100% EtOAc/hexanes) to afford **3 b** as yellow oil (20.98 g, 93%). ee = 98.5% (CHIRALPAK® AD–H column, CO_2/MeOH 5% to 50% in 8 min, flow rate 2 mL/min, temperature 30 °C). ^{1}H NMR ([D$_6$]DMSO, 500 MHz): δ = 8.74 (s, 1H), 8.34 (d, J = 6.1 Hz, 1H), 7.19 (d, J = 6.1 Hz, 1H), 4.02 (q, J = 7.2 Hz, 2H), 3.44–3.47 (m, 1H), 3.24–3.30 (m, 2H), 3.10–3.15 (m, 1H), 2.67–2.72 (m, 1H), 1.89–1.99 (m, 1H), 1.68–1.76 (m, 2H), 1.53–1.60 (m, 1H), 1.24 (t, J = 7.2 Hz, 3H); ^{13}C NMR ([D$_6$]

DMSO, 125 MHz): δ = 172.31, 152.33, 148.97, 147.51, 135.73, 113.54, 60.11, 50.80, 49.49, 40.09, 25.78, 23.08, 13.87. LRMS (ESI) *m/z* 280.1 $[M+H]^+$.

*(R)-Ethyl 1-(3-aminopyridin-4-yl)piperidine-3-carboxylate (**4b**):* To a solution of (*R*)-ethyl 1-(3-nitropyridin-4-yl) piperidine-3-carboxylate (**3b**; 12.11 g, 43.4 mmol) in EtOAc (100 mL) was added Pd/C (10 wt. % loading, 1.21 g). The resultant mixture was shaken under H_2 (50 psi) in a Parr hydrogenation flask for 3 h, and filtered through Celite. The filtrate was evaporated to give **4b** as brown oil (10.49 g, 97%). ^{1}H NMR ([D$_6$]DMSO, 500 MHz): δ = 7.90 (s, 1H), 7.72 (d, *J* = 5.2 Hz, 1 H), 6.77 (d, *J* = 5.2 Hz, 1 H), 4.92 (br s, 2H, NH_2), 4.07 (q, *J* = 7.1 Hz, 2H), 2.97–3.10 (m, 2H), 2.86–2.94 (m, 1H), 2.74–2.78 (m, 1H), 2.64–2.72 (m, 1H), 1.81–1.89 (m, 1H), 1.61–1.77 (m, 3H), 1.19 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): δ = 173.33, 143.96, 138.74, 137.99, 136.50, 113.48, 60.07, 50.69, 49.58, 40.58, 25.46, 23.87, 14.03. LRMS (ESI) *m/z* 250.1 $[M+H]^+$.

*3-Amino-6-(2-fluoro-5-isopropoxyphenyl)pyrazine-2-carboxylic acid (**8b**):* To a suspension of 3-amino-6-bromopyrazine-2-carboxylic acid (5; 872.0 mg, 4.0 mmol), (2-fluoro-5-isopropoxyphenyl)boronic acid (950.4 mg, 4.8 mmol), $Pd(PPh_3)_4$ (231.1 mg, 0.2 mmol) and Na_2CO_3 (1.27 g, 12.0 mmol) in dioxane (16 mL) was added water (4 mL) to result in a mixture which was heated at 90 °C for 3 h, concentrated. The residue was loaded on silica gel and subjected to chromatography (CH_2Cl_2/MeOH/28%NH_4OH, 40 : 20 : 1, *v/v/v*) to afford the amonium salt of the product which was then dissolved in water (160 mL), washed by EtOAc (2×20 mL) and adjusted to pH 2 by adding concentrated HCl solution. The solid precipitated was collected by filtration to afford **8b** (745.2 mg, 64%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 8.80 (s, 1H), 7.26 (dd, *J* = 6.3, 3.1 Hz, 1H), 7.10 (dd, *J* = 10.5, 9.0 Hz, 1H), 6.92 (ddd, *J* = 9.0, 4.0, 3.1 Hz, 1H), 4.54 (septet, *J* = 6.0 Hz, 1H), 1.36 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR ([D$_6$]DMSO, 125 MHz): δ = 167.60, 154.76 (J_{CF} = 3.1 Hz), 153.90, 153.78 (d, J_{CF} = 238 Hz), 147.45 (d, J_{CF} = 11.5 Hz), 135.44 (d, J_{CF} = 3.6 Hz), 124.53 (d, J_{CF} = 13.9 Hz), 123.15, 116.87 (d, J_{CF} = 24.7 Hz), 116.80 (d, J_{CF} = 6.7 Hz), 116.63 (d, J_{CF} = 2.9 Hz), 70.00, 21.78. LRMS (ESI) *m/z* 292.1 $[M+H]^+$.

*Ethyl (R)-1-(3-(3-amino-6-(2-fluoro-5-isopropoxyphenyl)pyrazine-2-carboxamido)pyridin-4-yl)piperidine-3-carboxylate (**7b**):* To a mixture of (*R*)-ethyl 1-(3-aminopyridin-4-yl) piperidine-3-carboxylate (**4b**; 565.9 mg, 2.27 mmol) and 3-amino-6-(2-fluoro-5-isopropoxyphenyl) pyrazine-2-carboxylic acid (**8b**; 701.7 mg, 2.73 mmol) in DMF (15 mL) was added EDCI (523.3 mg, 2.73 mmol) and HOAt (371.5 mg, 2.73 mmol). After the resulting mixture was stirred at 25 °C for 18 h, DMF was removed under reduced pressure, and the residue was purified by reversed-phase HPLC [10–70% MeCN (0.035% TFA)/water (0.05% TFA)]. After MeCN was removed from the desired fractions under reduced pressure, the residue was extracted with 5% MeOH in CH_2Cl_2 (3×30 mL). The combined organic extracts were washed with a saturated aqueous $NaHCO_3$ solution, dried over MgSO4 and evaporated to afford **7b** (853.5 mg, 72%). ^{1}H NMR ([D$_6$]DMSO, 500 MHz): δ = 10.27 (s, 1H, NH), 9.28 (s, 1H), 8.68 (d, *J* = 2.5 Hz, 1H), 8.27 (d, *J* = 5.3 Hz, 1H), 7.83 (br s, 2H, NH_2), 7.41 (dd, *J* = 6.5, 3.2 Hz, 1H), 7.25 (dd, *J* = 10.7, 9.0 Hz, 1H), 7.16 (d, *J* = 5.3 Hz, 1H), 7.03 (m, 1H), 4.64 (septet, *J* = 6.0 Hz, 1H), 3.88 (d, *J* = 7.1 Hz, 2H), 3.30–3.38 (m, 1H), 3.12–3.17 (m, 1H), 2.61–2.70 (m, 3H), 1.71–1.78 (m, 1H), 1.57–1.71 (m, 2H), 1.31–1.39 (m, 1H), 1.25 (d, *J* = 6.0 Hz, 6H), 1.00 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ([D$_6$]DMSO, 125 MHz): δ = 172.28, 163.33, 154.26, 153.90 (d, J_{CF} = 1.1 Hz), 153.62 (d, J_{CF} = 238.3 Hz), 149.44, 147.81 (d, J_{CF} = 10.2 Hz), 146.00, 141.70, 134.79 (d, J_{CF} = 3.3 Hz), 127.77, 124.53 (d, J_{CF} = 14.2 Hz), 123.68, 117.20 (d, J_{CF} = 2.4 Hz), 116.70 (d, J_{CF} = 24.2 Hz), 115.67 (d, J_{CF} = 8.0 Hz), 114.76, 69.75, 59,75, 52.70, 50.61, 41.32, 26.00, 24.38, 21.58, 13.68. LRMS (ESI) *m/z* 523.3 $[M+H]^+$.

*(R)-1-(3-(3-Amino-6-(2-fluoro-5-isopropoxyphenyl)pyrazine-2-carboxamido)pyridin-4-yl)piperidine-3-carboxylic acid (**1**):* To a solution of ethyl (*R*)-1-(3-(3-amino-6-(2-fluoro-5-isopropoxyphenyl) pyrazine-2-carboxamido)pyridin-4-yl)piperidine-3-carboxylate (**7b**; 156.7 mg, 0.3 mmol) in THF/MeOH (5 : 3, *v/v*, 8 mL) was added an aqueous solution of LiOH (1.0 M, 0.6 mL, 0.6 mmol). The resulting mixture was stirred at 25 °C for 4 h and concentrated under reduced pressure. The residue was dissolved in water (60 mL), washed with CH_2Cl_2 (10 mL) and then acidified with an aqueous HCl solution (1.0 M, 0.6 mL, 0.6 mmol). The solid precipitate was collected by filtration and dried under reduced pressure to afford **1** (150.4 mg, 96%). *ee* = 97.2% (CHIRALPAK® AD–H column, CO_2/MeOH + 0.5% diethylamine, 5% to 50% in 8 min, flow rate 2 mL/min, temperature 30 °C). ^{1}H NMR ([D$_6$]DMSO, 400 MHz): 10.30 (s, 1H, NH), 9.32 (s, 1H), 8.68 (d, *J* = 2.1 Hz, 1H), 8.27 (d, *J* = 5.3 Hz, 1H), 7.84 (br s, 2H, NH_2), 7.41 (dd, *J* = 6.5, 3.1 Hz, 1H), 7.25 (dd, *J* = 10.6, 9.1 Hz, 1H), 7.18 (d, *J* = 5.3 Hz, 1H), 7.00–7.15 (m, 1H), 4.64 (septet, *J* = 7.1 Hz, 1H), 3.26–3.33 (m, 1H), 3.11–3.17 (m, 1H), 2.67–2.73 (m, 1H), 2.53–2.60 (m, 2H), 1.73–1.81 (m, 1H), 1.57–1.66 (m, 2H), 1.26–1.37 (m, 1H), 1.26 (d, *J* = 7.1 Hz, 6H); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): δ = 174.42, 163.38, 154.29, 153.97, 153.65 (d, J_{CF} = 238 Hz), 149.69, 147.87 (d, J_{CF} = 10.4 Hz), 145.97, 134.84 (d, J_{CF} = 3.2 Hz), 128.00, 124.52 (d, J_{CF} = 14.0 Hz), 123.71, 117.19, 116.82 (d, J_{CF} = 24.2 Hz), 115.72 (d, J_{CF} = 7.9 Hz), 114.89, 69.85, 52.97, 51.10, 41.48, 26.30, 24.61, 21.60. LRMS (ESI) *m/z* 495.2 $[M+H]^+$. Anal. calcd. (%) for $C_{25}H_{27}FN_6O_4$: C 60.72, H 5.50, N 16.99; found: C 60.60, H 5.46, N 16.88; purity ≥ 99.8%.

*3-Amino-*N*-(4-(4-aminopiperidin-1-yl)pyridin-3-yl)-6-phenylpyrazine-2-carboxamide (**9**):* ^{1}H NMR (CD_3OD, 400 MHz): δ = 9.41 (s, 1H), 8.77 (s, 1H), 8.23 (d, *J* = 5.4 Hz, 1H), 8.01–8.04 (dd, *J* = 8.0, 1.3 Hz, 2H), 7.52–7.58 (dd, *J* = 8.0, 7.3 Hz, 2H), 7.42–7.49 (m, 1H), 7.21 (d, *J* = 5.4 Hz, 1H), 3.20–3.27 (m, 2H), 2.78–2.81 (m, 2H), 2.60–2.68 (m, 1H), 1.82–1.92 (m, 2H), 1.44–1.55 (m, 2H). LRMS (ESI) *m/z* 390.2 $[M+H]^+$.

*1-(4-(3-Amino-6-phenylpyrazine-2-carboxamido)pyridin-3-yl)piperidine-4-carboxylic acid (**11**):* ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 1.11 (s, 1H, NH), 9.05 (s, 1H), 8.65–8.71 (m, 2H), 8.52–8.60 (m, 1H), 8.10 (d, *J* = 7.2 Hz, 2H), 7.86 (br s, 2H, NH_2), 7.48–7.52 (m, 2H), 7.39 (m, 1H), 3.09–3.16 (m, 2H), 2.86–2.89 (m, 2H), 2.38–2.46 (m, 1H), 1.97–2.05 (m, 2H), 1.77–1.86 (m, 2H). LRMS (ESI) *m/z* 419.2 $[M+H]^+$.

*1-(2-(3-Amino-6-phenylpyrazine-2-carboxamido)pyridin-3-yl)piperidine-4-carboxylic acid (**12**):* ^{1}H NMR (CD_3OD, 400 MHz): δ = 8.90 (s, 1H), 8.16–8.23 (m, 2H), 8.02 (d, *J* = 7.3 Hz, 2H), 7.49–7.53 (m, 3H), 7.37–7.44 (m, 1H), 3.17–3.25 (m, 2H), 2.86–2.95 (m, 2H), 2.46–2.52 (m, 1H), 2.08–2.18 (m, 2H), 1.91–2.03 (m, 2H). LRMS (ESI) *m/z* 419.2 $[M+H]^+$.

*1-(3-(3-Amino-6-phenylpyrazine-2-carboxamido)pyridin-2-yl)piperidine-4-carboxylic acid (**13**):* ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 10.53 (s, 1H, NH), 8.99 (s, 1H), 8.67 (dd, *J* = 8.0, 1.6 Hz, 1H), 8.11–8.13 (m, 3H), 7.79 (br s, 2H, NH_2), 7.47 (dd, *J* = 7.9, 7.4 Hz, 2H), 7.38 (dd, *J* = 7.4, 6.4 Hz, 1H), 7.21 (dd, *J* = 8.0, 4.8 Hz, 1H), 3.84–3.87 (m, 2H), 3.26–3.32 (m, 2H), 2.36–2.43 (m, 1H), 2.10–2.28 (m, 2H), 1.96–2.03 (m, 2H). LRMS (ESI) *m/z* 419.2 $[M+H]^+$.

*1-(2-(3-Amino-6-phenylpyrazine-2-carboxamido)phenyl)piperidine-4-carboxylic acid (**14**):* ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 10.82 (s, 1H, NH), 8.96 (s, 1H), 8.45 (dd, *J* = 8.0, 1.6 Hz, 1H), 8.11 (dd, *J* = 8.0, 1.3 Hz, 2H), 7.79 (br s, 2H, NH_2), 7.49 (dd, *J* = 7.8, 7.4 Hz, 2H), 7.37 (dd, *J* = 7.4, 7.0 Hz, 1H), 7.33 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.11–7.21 (m, 2H), 2.95–3.04 (m, 2H), 2.70–2.80 (m, 2H), 2.33–2.42 (m, 1H), 1.95–2.04 (m, 2H), 1.78–1.90 (m, 2H). LRMS (ESI) *m/z* 418.2 $[M+H]^+$.

*1-(3-(3-Amino-6-phenylpyrazine-2-carboxamido)pyridin-4-yl)azetidine-3-carboxylic acid (**15**):* LRMS (ESI) *m/z* 391.2 $[M+H]^+$.

*1-(3-(3-Amino-6-(pyridin-4-yl)pyrazine-2-carboxamido)pyridin-4-yl)pyrrolidine-3-carboxylic acid (**16**):* LRMS (ESI) m/z 405.2 $[M+H]^+$.

*1-(3-(3-Amino-6-phenylpyrazine-2-carboxamido)pyridin-4-yl)-4-fluoropiperidine-4-carboxylic acid (**17**):* ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 10.45 (s, 1H, NH), 8.98 (s, 1H), 8.80 (br s, 1H), 8.36 (d, J = 6.3 Hz, 1H), 8.18 (d, J = 7.3 Hz, 2H), 7.71 (br s, 2H, NH$_2$), 7.47 (dd, J = 7.7, 7.3 Hz, 2H), 7.37–7.44 (m, 2H), 3.84–3.87 (m, 2H), 3.26–3.32 (m, 2H), 2.10–2.28 (m, 2H), 1.96–2.03 (m, 2H). LRMS (ESI) m/z 437.2 [M + H]$^+$.

*(S)-1-(3-(3-Amino-6-phenylpyrazine-2-carboxamido)pyridin-4-yl)piperidine-3-carboxylic acid (**18**):* ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 10.49 (s, 1H, NH), 9.38 (s, 1H), 8.98 (s, 1H), 8.29 (d, J = 5.3 Hz, 1H), 8.12 (dd, J = 8.5, 1.3 Hz, 2H), 7.78 (br s, 2H, NH$_2$), 7.48–7.53 (m, 2H), 7.41–7.48 (m, 1H), 7.24 (J = 5.3 Hz, 1H), 3.27–3.36 (m, 1H), 3.04–3.12 (m, 1H), 2.77–2.83 (m, 1H), 2.65–2.74 (m, 1H), 2.52–2.61 (m, 1H), 1.78–1.81 (m, 1H), 1.60–1.71 (m, 2H), 1.37–1.43 (m, H). LRMS (ESI) m/z 419.2 [M + H]$^+$.

*(R)-1-(3-(3-Amino-6-phenylpyrazine-2-carboxamido)pyridin-4-yl)piperidine-3-carboxylic acid (**19**):* ^{1}H NMR ([D$_6$]DMSO, 500 MHz) of the sodium salt: δ 10.56 (s, 1H, NH), 9.40 (s, 1H), 8.99 (s, 1H), 8.27 (d, J = 5.3 Hz, 1H), 8.16 (dd, J = 8.5, 1.3 Hz, 2H), 7.76 (br s, 2H, NH$_2$), 7.51 (dd, J = 7.9, 7.4 Hz, 2H), 7.39–7.45 (m, 1H), 7.19 (J = 5.3 Hz, 1H), 3.32–3.30 (m, 2H), 3.02–3.10 (m, 1H), 2.60–2.66 (m, 1H), 2.27–2.35 (m, 1H), 1.84–1.92 (m, 1H), 1.57–1.70 (m, 2H), 1.23–1.29 (m, 1H). LRMS (ESI) m/z 419.2 [M + H]$^+$.

*(R)-4-(3-(3-Amino-6-phenylpyrazine-2-carboxamido)pyridin-4-yl)piperazine-2-carboxylic acid (**20**):* ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 10.37 (s, 1H, NH), 9.26 (s, 1H), 8.99 (s, 1H), 8.30 (d, J = 5.3 Hz, 1H), 8.14 (dd, J = 7.3, 1.1 Hz, 2H), 7.77 (br s, 2H, NH$_2$), 7.52 (dd, J = 7.8, 7.3 Hz, 2H), 7.40–7.47 (m, 1H), 7.23 (J = 5.3 Hz, 1H), 3.14–3.51 (m, 4H), 2.86–2.97 (m, 2H), 2.77–2.82 (m, 1H). LRMS (ESI) m/z 420.2 [M + H]$^+$.

*(R)-2-(1-(3-(3-Amino-6-phenylpyrazine-2-carboxamido)pyridin-4-yl)piperidin-3-yl)acetic acid (**21**):* ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 10.50 (s, 1H, NH), 8.99 (s, 1H), 8.72 (s, 1H), 8.36 (d, J = 6.9 Hz, 1H), 8.21 (dd, J = 7.3, 1.3 Hz, 2H), 7.69 (br s, 2H, NH$_2$), 7.50 (dd, J = 7.8, 7.3 Hz, 2H), 7.37–7.43 (m, 2H), 3.87–3.96 (m, 2H), 3.02–3.10 (m, 1H), 2.90–2.99 (m, 1H), 2.01–2.19 (m, 3H), 1.70–1.77 (m, 1H), 1.62–1.70 (m, 1H), 1.50–1.59 (m, 1H), 1.19–1.26 (m, 1H). LRMS (ESI) m/z 433.2 [M + H]$^+$.

*(S)-2-(1-(3-(3-Amino-6-phenylpyrazine-2-carboxamido)pyridin-4-yl)piperidin-3-yl)acetic acid (**22**):* ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 10.50 (s, 1H, NH), 8.99 (s, 1H), 8.72 (s, 1H), 8.36 (d, J = 6.9 Hz, 1H), 8.21 (dd, J = 7.3, 1.3 Hz, 2H), 7.69 (br s, 2H, NH$_2$), 7.50 (dd, J = 7.8, 7.3 Hz, 2H), 7.38–7.43 (m, 2H), 3.87–3.96 (m, 2H), 3.01–3.10 (m, 1H), 2.90–2.98 (m, 1H), 1.99–2.18 (m, 3H), 1.70–1.77 (m, 1H), 1.59–1.67 (m, 1H), 1.50–1.57 (m, 1H), 1.18–1.26 (m, 1H). LRMS (ESI) m/z 433.2 [M + H]$^+$.

*1-(3-(3-Amino-6-(2-fluorophenyl)pyrazine-2-carboxamido)pyridin-4-yl) piperidine-4-carboxylic acid (**23**):* ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 9.17 (s, 1H), 8.71 (d, J = 2.3 Hz, 1H), 8.28 (d, J = 6.7 Hz, 1H), 7.91 (ddd, J = 7.8, 7.8, 1.8 Hz, 1H), 7.45 (d, J = 6.8 Hz, 1H), 7.39–7.45 (m, 1H), 7.31 (ddd, J = 7.6, 7.5, 1.1 Hz, 1H), 7.21 (ddd, J = 11.4, 8.3, 0.9 Hz, 1H), 3.75–3.81 (m, 2H), 3.13–3.21 (m, 2H), 2.49–2.55 (m, 1H), 2.01–2.09 (m, 2H), 1.81–1.91 (m, 2H). LRMS (ESI) m/z 437.2 [M + H]$^+$.

*1-(3-(3-Amino-6-(o-tolyl)pyrazine-2-carboxamido)pyridin-4-yl)piperidine-4-carboxylic acid (**24**):* ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 9.23 (s, 1H), 8.43 (s, 1H), 8.28 (d, J = 6.2 Hz, 1H), 7.44 (d, J = 6.5 Hz, 1H), 7.39 (d, J = 6.8 Hz, 1H), 7.26–7.35 (m, 3H), 3.66–3.71 (m, 2H), 3.09–3.16 (m, 2H), 2.43 (s, 1H), 2.39 (s, 3H), 1.90–1.98 (m, 2H), 1.68–1.78 (m, 2H). LRMS (ESI) m/z 433.2 [M + H]$^+$.

*1-(3-(3-Amino-6-(2-methoxyphenyl)pyrazine-2-carboxamido)pyridin-4-yl)piperidine-4-carboxylic acid (**25**):* ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 9.14 (s, 1H), 8.79 (s, 1H), 8.29 (d, J = 7.6 Hz, 1H), 7.76 (dd, J = 7.6, 1.7 Hz, 1H), 7.47 (d, J = 6.9 Hz, 1H), 7.40 (ddd, J = 7.4, 6.6, 1.8 Hz, 1H), 7.12 (d, J = 8.3 Hz, 1H), 7.08 (ddd, J = 7.5, 7.5, 0.9 Hz, 1H), 3.90 (s, 3H), 3.79–3.84 (m, 2H), 3.15–3.22 (m, 2H), 2.49–2.55 (m, 1H), 1.98–2.06 (m, 2H), 1.78–1.88 (m, 2H). LRMS (ESI) m/z 449.2 [M + H]$^+$.

*1-(3-(3-Amino-6-(2-(trifluoromethyl)phenyl)pyrazine-2-carboxamido) pyridin-4-yl)piperidine-4-carboxylic acid (**26**):* ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 9.28 (s, 1H), 8.42 (s, 1H), 8.28 (d, J = 6.0 Hz, 1H), 7.82 (d, J = 7.6 Hz, 1H), 7.71 (dd, J = 7.4, 7.3 Hz, 1H), 7.62 (dd, J = 7.7, 7.6 Hz, 1H), 7.56 (J = 7.6 Hz, 1H), 7.42 (d, J = 6.8 Hz, 1H), 3.65–3.69 (m, 2H), 3.01–3.08 (m, 2H), 2.36–2.42 (m, 1H), 1.84–1.90 (m, 2H), 1.57–1.67 (m, 2H). LRMS (ESI) m/z 487.2 [M + H]$^+$.

*1-(3-(3-Amino-6-(4-fluorophenyl)pyrazine-2-carboxamido)pyridin-4-yl) piperidine-4-carboxylic acid (**27**):* ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 9.12 (s, 1H), 8.76 (s, 1H), 8.28 (dd, J = 6.8, 1.9 Hz, 1H), 7.96 (dd, J = 8.9, 8.6 Hz, 2H), 7.45 (d, J = 6.8 Hz, 1H), 7.19 (dd, J = 8.7, 8.6 Hz, 2H), 3.79–3.83 (m, 2H), 3.16–3.24 (m, 2H), 2.54–2.60 (m, 1H), 2.04–2.12 (m, 2H), 1.86–1.97 (m, 2H). LRMS (ESI) m/z 437.2 [M + H]$^+$.

*1-(3-(3-Amino-6-(*p*-tolyl)pyrazine-2-carboxamido)pyridin-4-yl)piperidine-4-carboxylic acid (**28**):* ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 9.08 (d, J = 0.9 Hz, 1H), 8.78 (s, 1H), 8.31 (dd, J = 6.8, 1.1 Hz, 1H), 7.89 (d, J = 8.1 Hz, 2H), 7.45 (d, J = 6.9 Hz, 1H), 7.29 (d, J = 8.1 Hz, 2H), 3.82–3.88 (m, 2H), 3.16–3.22 (m, 2H), 2.54–2.60 (m, 1H), 2.39 (s, 3H), 2.02–2.09 (m, 2H), 1.84–1.95 (m, 2H). LRMS (ESI) m/z 433.2 [M + H]$^+$.

*1-(3-(3-Amino-6-(4-(trifluoromethyl)phenyl)pyrazine-2-carboxamido) pyridin-4-yl)piperidine-4-carboxylic acid (**29**):* ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 8.91 (s, 1H), 8.89 (d, J = 1.0 Hz, 1H), 8.23–8.30 (m, 3H), 7.79 (d, J = 8.2 Hz, 2H), 7.45 (d, J = 6.9 Hz, 1H), 3.93–4.02 (m, 2H), 3.21–3.30 (m, 2H), 2.59–2.66 (m, 1H), 2.01–2.11 (m, 2H), 1.85–1.96 (m, 2H). LRMS (ESI) m/z 487.2 [M + H]$^+$.

*1-(3-(3-Amino-6-(3-methoxyphenyl)pyrazine-2-carboxamido)pyridin-4-yl)piperidine-4-carboxylic acid (**30**):* ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 9.08 (d, J = 0.9 Hz, 1H), 8.81 (s, 1H), 8.30 (dd, J = 6.9, 1.0 Hz, 1H), 7.53–7.60 (m, 2H), 7.48 (d, J = 6.9 Hz, 1H), 7.39 (dd, J = 8.1, 7.8 Hz, 1H), 6.97 (dd, J = 7.8, 2.3 Hz, 1H), 3.81–3.85 (m, 2H), 3.18–3.24 (m, 2H), 2.56 (m, 1H), 2.01–2.10 (m, 2H), 1.85–1.96 (m, 2H). LRMS (ESI) m/z 449.2 [M + H]$^+$.

*1-(3-(3-Amino-6-(m-tolyl)pyrazine-2-carboxamido)pyridin-4-yl)piperidine-4-carboxylic acid (**31**):* ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 9.03 (d, J = 1.0 Hz, 1H), 8.80 (s, 1H), 8.30 (dd, J = 6.9, 1.2 Hz, 1H), 7.84 (s, 1H), 7.80 (d, J = 7.9 Hz, 1H), 7.47 (d, J = 6.9 Hz, 1H), 7.39 (dd, J = 7.7, 7.6 Hz, 1H), 7.18–7.24 (m, 1H), 3.85–3.93 (m, 2H), 3.18–3.28 (m, 2H), 2.54–2.60 (m, 1H), 2.43 (s, 3H), 2.01–2.09 (m, 2H), 1.84–1.94 (m, 2H). LRMS (ESI) m/z 433.2 [M + H]$^+$.

*1-(3-(3-Amino-6-(2-fluoro-5-(trifluoromethoxy)phenyl)pyrazine-2-carboxamido)pyridin-4-yl)piperidine-4-carboxylic acid (**32**):* ^{1}H NMR (CD$_3$OD, 400 MHz): δ = 8.95 (d, J = 1.0 Hz, 1H), 8.77 (d, J = 2.0 Hz, 1H), 8.28 (d, J = 6.9 Hz, 1H), 7.98 (d, J = 6.3 Hz, 1H), 7.45 (d, J = 6.9 Hz, 1H), 7.37 (d, J = 6.9 Hz 2H), 3.89–3.98 (m, 2H), 3.21–3.28 (m, 2H), 2.54–2.60 (m, 1H), 1.98–2.07 (m, 2H), 1.75–1.85 (m, 2H). LRMS (ESI) m/z 503.2 [M + H]$^+$.

(R)-1-(3-(3-Amino-6-(2-fluoro-5-(trifluoromethoxy)phenyl)pyrazine-2-carboxamido)pyridin-4-yl)piperidine-3-carboxylic acid (**33**): ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 10.35 (s, 1H, NH), 9.18 (s, 1H), 8.73 (d, J = 2.4 Hz, 1H), 8.23 (d, J = 5.4 Hz, 1H), 8.00 (dd, J = 7.0, 2.4 Hz, 1H), 7.93 (br s, 2H, NH$_2$), 7.48–7.54 (m, 2H), 7.09 (d, J = 5.4 Hz, 1H), 3.21–3.28 (m, 1H), 3.11–3.17 (m, 1H), 2.52–2.59 (m, 2H), 2.00–2.07 (m, 1H), 1.71–1.78 (m, 1H), 1.43–1.62 (m, 2H), 1.19–1.25 (m, 1H). LRMS (ESI) m/z 521.2 [M + H]$^+$.

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Conflict of Interest

The authors declare no conflict of interest.

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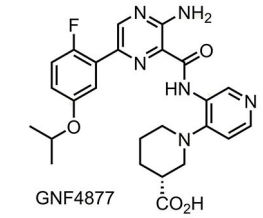
FULL PAPERS

Getting more cells on site: Promoting β-cell proliferation with inhibitors of dual-specificity tyrosine-regulated kinase 1A (DYRK1A) offers the potential for treating diabetes patients by restoring β-cell mass. Lead optimization of an aminopyrazine hit led to the identification of GNF4877, a potent dual inhibitor of DYRK1A and glycogen synthase kinase 3β (GSK3β).



GNF4877

DYRK1A IC_{50} = 0.006 μM
GSK3β IC_{50} = 0.016 μM
rat β cell = 0.19 μM (162% eff)
human β cell = 0.54 μM (263% eff)

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