Supporting information:

New β-alanine Derivatives are Orally Available Glucagon Receptor Antagonists:

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Synthesis

^{1}H-NMR spectra were recorded in deuterated solvents at 200, 300 or 400 MHz (DRX 200, DRX 300 and AMX2 400, from Bruker Instruments, respectively). Chemical shifts are reported as δ values (ppm) relative to internal tetramethylsilane (δ = 0 ppm). Elemental analyses were performed by the microanalytical laboratories at Novo Nordisk A/S, Denmark and SDU, Denmark. Column chromatography[29] was performed on silica gel 60 (40-63 μm). Melting points were determined in open capillary tubes on a Büchi 535 apparatus and are uncorrected. Chemicals and solvents used were commercially available and were used without further purification. Yields refer to pure materials and are not optimized.

Pharmacodynamic models

A. Glucagon challenged rat.

Non-fasted male Sprague Dawley rats (200 g) were maintained in the anaesthetized state during the test by s.c. administration of a 1:1 mixture of Hypnorm (fentanyl, 0.05 mg/mL and fluanizone, 2.5 mg/mL, Janssen Pharma Ldt, Copenhagen, Denmark) and Dormicum (Midazolam, 1.25 mg/mL, Roche, Basel, Switzerland). A catheter was inserted in a jugular vein for administration of compounds. Approximately 60 min after initiation of anesthesia, test compounds (0, 1, 3, 10 and 30mg/kg) and glucagon (3 μg/kg) were administered in 5 min intervals, respectively. Samples for determination of blood glucose concentrations were taken from the tail tip 25 and 5 min prior to

administration of the compound to represent average basal values and again 10 min after administration of glucagon (time for peak response of glucagon). The results were expressed as delta values calculated as the value obtained 10 min after glucagon administration minus the average of the two basal values.

B. Effect on blood glucose in ob/ob mice

Male ob/ob mice of the Umeå strain (11-13 weeks of age) used for the studies were selected from a larger group of mice having the highest blood glucose (BG) levels. Selected mice were randomised into treatment groups having matching BG levels. Compound **57** (0 and 100 mg/kg) was administered orally by gavage in the morning to non-fasted mice and blood samples (5 µl) for determination of BG were obtained ca. 30 min pre-dose and at 2, 4, 6 and 24 hour post-dose. Mice had no access to food the first 6 hours after dosing, and subsequently had free access to food. Food and water intake were measured in the 6-24 hour post-dose interval.

Incubation with rat liver microsomes

Metabolic rate: Incubation time: 0, 5, 10, 10, 30 min (n=3), total incubation volume: 150 µL, protein content: 0.331 mg/ml, compound concentration: 10 µM. Incubation conditions: 37°C, UDP-GA: 1 mM, NADPH: 1 mM, KH_2PO_4 (pH 7.4) buffer up to 150 µL. All incubations were performed in a 96 well plate format and a Packard liquid handler was applied for incubations and liquid handling. Microsomal incubations were terminted by applying 150 µL of MeCN. The metabolic rates and metabolic profiles were determined by LC-MS.

Metabolic profiling

Metabolic rate: Incubation time: 0, 60 min, (n =3 for LC-MS analysis and n= 5 for LC-NMR analysis), total incubation volume: 1000 µL, protein content: 1 mg/mL, NCE concentration: 25 µM, Incubation conditions: 37°C UDP-GA: 1 mM, NADPH: 1 mM, KH_2PO_4 (pH 7.4) buffer up to 1000 µL.

Microsomal incubations were terminated by applying the samples to solid phase extraction (SPE).

SPE method

SPE column: SPEC (C_8) SPE cartridge, Activation: 1000 µL MeOH + 1000 µL $NaHPO_4$ (pH 7.4), Sample volume: 1000 µL, Washing: 2 X 1000 µL $NaHPO_4$ (pH 7.4)

Eluate: 1000 µL MeOH , The 3 samples (LC-MS) or 5 samples (LC-NMR) at each time point were pooled and the solvent evaporated under N_2-gas at 40°C. 250 µL of mobile phase was then added to the evaporated sample and analyzed by LC-MS or LC-NMR.

Chromatography

The HPLC system used for the directly-coupled HPLC-NMR experiment consisted of a Hewlet Packard 1050 series chromatograph (Palo Alto, California, USA) where the variable wavelength UV-detector was operated at 340 nm. The chromatographic system was connected to a Bruker BPSU-12 collector (Rheinstetten, Germany) and the chromatography was controlled by Bruker Hystar software. The BPSU-12 collector was connected to the HPLC-NMR flow-probe *via* an inert polyetherketone capillary (0.25 mm I.D.). The chromatographic data