SUPPLEMENTARY INFORMATION

To determine rat hemoglobin A1c levels, blood cells were lysed and the proteins de-salted using 100 μl OMIX C4 tips (Agilent Technologies, Santa Clara, CA) using the manufacturer’s protocol with 10 loading cycles and elution with 10 μl 75% MeOH. Mass spectra were acquired on a mass spectrometer (Bruker Daltonics, Bremen, Germany). After desalting, 5 μl of each tip eluate was diluted with 95 μl H2O:MeOH 1:1 (v/v) and 0.1% formic acid and infused directly into the electrospray source at 2 μl/min using the built-in syringe pump and a 100 μl syringe. For HbA1c quantitation, 64 full profile spectra of 1 M data points (1.9 s transient) covering m/z 829-1,000 were averaged, zero-filled (1X) and sine square apodized (1). Quadrupole transmission was optimized at the mid-point of the m/z range. The resolving power (m/Δm, FWHM) of the hemoglobin chains was ~150,000. Wider range spectra (m/z 598-2,000) were also acquired to characterize the protein contents of the sample and the charge state distributions of the different hemoglobin chains. All spectra were acquired in broadband mode and externally calibrated using the heme monomer and dimer peaks. After conversion of full profile data to mzXML (2), Xtractor (3) was used to quantify the six most abundant isotopic peaks of the [M+18H]18+ α-chain (UniProtKB HBA_RAT, m/z ~845.3), β-chain (UniProtKB HBB1_RAT, putatively with the T124S mutation, m/z ~880.6), the same β-chain with a glucose adduct (m/z ~889.6) and corresponding background signal from an empty region of the spectra. The fraction of glucose adduct was reported as the ratio of the signal of the β-chain with a glucose adduct to the total β-chain signal.

REFERENCES

Supplementary Figure S2. Effect of ARA290 on i.p. glucose tolerance test (IPGTT) in Wistar rats. (A) Baseline IPGTT in Wistar rats before treatment with ARA290 (n=6) or PBS (n=6). (B) IPGTT after 2 wks of treatment with ARA290 (n=6) or PBS (n=6) in Wistar rats. (C) IPGTT after 2 wks of treatment with ARA290 (n=6) or PBS (n=6) in Wistar rats. Plasma glucose levels are represented as means ± SEM. (● ARA290-treated, ○ placebo-treated).

Supplementary Figure S3. ARA290 does not effect insulin sensitivity. (A) Effect on s.c. insulin tolerance test (SCITT) after 4 wks of treatment with ARA290 (n=8) or PBS (n=7) in GK rats. Plasma glucose levels are represented as means ± SEM. (● ARA290-treated, ○ placebo-treated). (B) Effect on i.p. pyruvate tolerance test (IPPTT) after 4 wks of treatment with ARA290 (n=8) or PBS (n=7) in GK rats. Plasma glucose levels are presented as means ± SEM. (● ARA290-treated, ○ placebo-treated).