Title: Non-peptidyl small molecule, adenosine, 5'-Se-methyl-5'-seleno-, 2',3'-diacetate, activates insulin receptor and attenuates hyperglycemia in type 2 diabetic *Lepr*$^{db/db}$ mice

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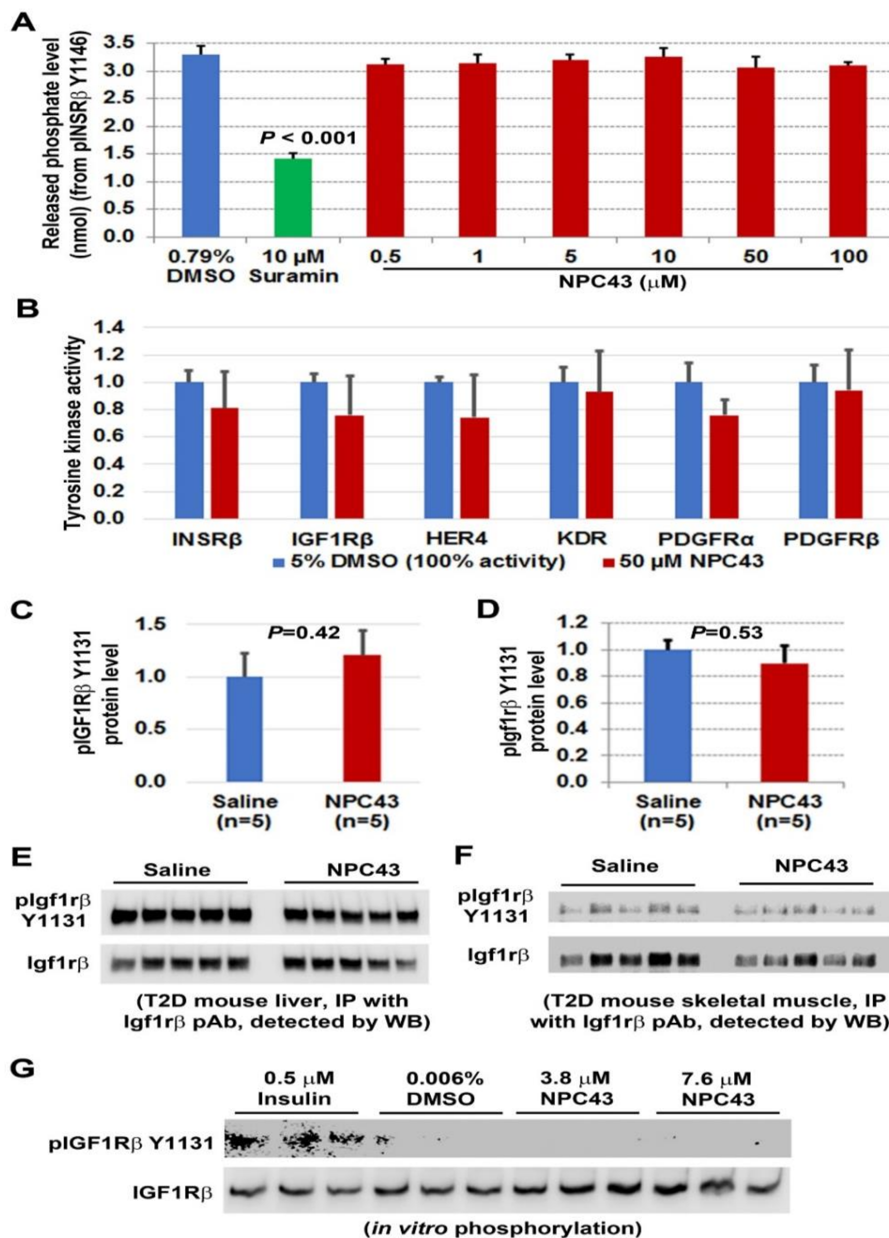
Supplementary Material-10



Effects of NPC43 on (A-B) the activities of intracellular PTP1B and several major tyrosine kinases *in vitro*, (C-E) the activation of endogenous Igf1r in liver and skeletal muscle of *Lepr$^{db/db}$* mice, and (G) the activation of IGF1R protein in a cell-free *in vitro* phosphorylation system. (A) No effects of NPC43 on the PTP1B activity *in vitro*. Recombinant PTP1B (2.5 ng) and its substrate (75 μM, INSRβ amino acid residues 1142-1153, pY-1146) were incubated with 0.79% (v/v) DMSO (the NPC43 solvent), suramin (a positive inhibitor, 10 μM) or 1-100 μM NPC43 and subjected to *in vitro* PTP1B assays to determine the released phosphate levels. Mean ± SD of triplicates per group. *P* values (vs 0.79%DMSO group) in all NPC43-treated groups was higher than 0.05 (*Student's t-test*). **(B)** No enhanced tyrosine kinase activity of INSRβ, IGF1Rβ, HER4, KDR, PDGFRα or PDGFRβ *in vitro* after NPC43 treatment, as determined by *in vitro* receptor tyrosine kinase assays. The NPC43 solvent [5% (v/v) DMSO in PBS buffer) was included as a positive control (referred to 100% kinase activity). Data are presented as mean ± SD of triplicates per group. *P* values in all NPC43-treated groups were higher than 0.05 (vs. the 5% DMSO group, *Student's t-test*). **(C-F)** No significant increase in protein levels of pIgf1rβ at Y1131 in the **(C, E)** liver and **(D, F)** gastrocnemius of *Lepr$^{db/db}$* mice after chronic i.p. treatment with NPC43, as determined by **(C-D)** ELISA and **(E-F)** co-immunoprecipitation analysis. Male *Lepr$^{db/db}$* mice at postnatal day 38 were i.p. injected daily with 0.2% (v/v) DMSO/physiological saline (referred to as the saline group) or NPC43 (0.136 mg/kg BW) for 52 days. Liver protein extracts (50 μg protein) or skeletal muscle protein extracts (150 μg protein) from five saline- or NPC43-treated mice were subjected to **(C-D)** ELISA analysis of pIgf1rβY1131**.** Data in **C-D** are presented as mean ± SEM, and *P* values between saline control and NPC43-treated groups were determined by performing *Student's t-test*. In **(E-F)**, liver or skeletal muscle protein extracts (600 μg) from five saline- or NPC43-treated mice were immunoprecipitated with a specific Igf1r antibody followed by Western blot analysis of pIgf1rβ at Y1131 and total Igf1rβ in these immunoprecipitated samples. **(G)** No activation of IGF1R protein by NPC43 in the cell-free *in vitro* phosphorylation system. Equal amounts of purified IGF1R protein (400 ng, triplicates per group) were incubated with 0.5 μM insulin, 0.006% (v/v) DMSO or NPC43 (3.8 or 7.6 μM) and subjected to *in vitro* phosphorylation analysis. Activated IGF1R (i.e. IGF1Rβ Y1131) and total IGF1Rβ protein in the *in vitro* phosphorylation reactions were detected by Western blot analysis. Experiments were repeated three times. Note the activation of IGF1R by insulin, but not by NPC43 at the tested doses.