Supporting Information

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SI Materials and Methods

Determination of Sterol and Fatty Acid Synthesis in Rat Primary Hepatocytes. Rat primary hepatocytes were seeded in six-well plates in hepatocyte attachment medium (Invitrogen) with 10% FBS (Atlanta Biologicals) overnight at a density of 0.75×10^6 cells per well. Medium was removed and the cells were washed once with PBS containing Ca^{2+} and Mg^{2+}. Medium was replaced with DMEM (Invitrogen) with high glucose, L-glutamine, and pyruvate pyridoxine hydrochloride. Compound or DMSO (Sigma) was added to each well and preincubated for 1 h. Lipid synthesis was monitored by adding into each well [^{14}C]acetate (0.6 μCi, Perkin-Elmer) for 2 h. Medium was then removed and the cells were washed again with cold PBS. To each well, 10% KOH in methanol (2.5 mL) and distilled water (1.0 mL) were then added. The suspension was then transferred into a new test tube and heated at 90 °C for 3 h, and lipid was extracted once with petroleum ether (4 mL). The upper petroleum ether layer (3 mL) was then transferred into scintillation vials and dried to monitor sterol synthesis. To determine fatty acid synthesis, the aqueous layer (3 mL) was extracted further. Platensimycin (PTM), cerulenin, C75, and TOFA [5-(tetradecyloxy)-2-furoic acid] were all tested under the same conditions. Platensimycin was used at 0.01, 0.1, 1, 10, and 100 μM final concentration; for C75 and TOFA, 0.1, 1, 10, and 100 μM; and cerulenin, 5, 40, and 200 μM.

Fatty Acid Oxidation Assay of Rat Primary Hepatocytes. Rat primary hepatocytes were seeded in six-well plates in attachment medium (2 mL) containing 10% FBS overnight at a density of 0.75×10^6 cells/well. Cells were washed once and transferred to medium 199 (Invitrogen) containing 1% Hepes (1 M), 20 mM glucose, 50 μM L-carnitine, and 0.25% BSA (free fatty acid-free) for 3 h. Compound was then added to each well for 30 min at 37 °C; 2.5 μL [^{14}C]oleic acid (0.1 μCi/μL) was diluted fourfold with incubation medium and added to each well and mixed. Each well was then gassed with O_2/CO_2 (95%/5%). The plate was sealed with parafilm and incubated for another hour. Medium (1 mL) was then transferred to a fresh tube containing 10% BSA (100 μL) to which 60% $HClO_4$ (100 μL) was added and mixed, and the content was spun at 12,000 rpm for 5 min. Supernatant (1 mL) was then transferred to a scintillation vial for counting.

Tissue Distribution of Platensimycin. Platensimycin was dosed to 11-wk-old *db/db* mice (n = 3 per time point) by oral gavage at 10, 30, and 100 mg/kg (mpk). Plasma, brain, as well as liver were collected at 0.5, 1, 3, 8, and 24 h after dosing. Concentrations of PTM in plasma, brain, and liver were determined by LC-MS/MS following methanol protein precipitation. The lower limit of quantitation of PTM in plasma was 1 ng/mL (0.0023 μM), whereas that for brain and liver was 8 ng/mL (0.0181 μM). All animal procedures were performed in accordance with the guidelines of the institutional animal care and use committee of Merck. Mice were group-housed and allowed ad libitum access to diet and autoclaved water. Animal-housing rooms were maintained at a constant room temperature (25 °C) in a 12-h light (7:00 AM)/dark (7:00 PM) cycle.

Relationship Between Liver Compound Levels and Inhibition of de Novo Lipogenesis. Different cohorts of mice were used to determine the effects of PTM on de novo lipid synthesis and its exposure in liver. Eleven to 14-wk-old *db/db* mice were used in pharmacodynamic assays (n = 5 mice per time point) or pharmacokinetic assays (3 mice per time point). For the pharmacodynamic assay, [^{3}H]H_2O (5 mL, 1 mCi/mL) was mixed with PBS (20 mL). Each *db/db* mouse received an oral gavage of either vehicle (0.5% methylcellulose) or compound. At 1, 3, and 7 h postdosing, each mouse received an i.p. injection of [^{3}H]H_2O at a dose of 1% vol/wt (e.g., 0.5 mL/50 g mouse). At 1 h after [^{3}H]H_2O administration, mice were euthanized and the liver was excised and frozen by using a freeze clamp precooled in liquid nitrogen.

Liver (~800 mg) was resuspended in 4 M KOH (1.5 mL) and 95% ethanol (1.5 mL) and extracted for [^{3}H]free fatty acid. The lower aqueous layer was acidified, fatty acids were extracted with hexane (4 mL), and centrifuged at 1,000 × g. The top hexane layer was back-washed and transferred into a counting vial and dried under a stream of warm air before counting.

Acute Effect of Platensimycin on Plasma D-β-Hydroxybutyrate Levels in *db/db* Mice. Fourteen-week-old *db/db* mice were fasted for 6 h and then dosed with PTM at 10, 30, and 100 mpk. Plasma was collected at 2 h postdose (n = 5 per group).

Determination of Malonyl-CoA Levels in Liver and Hypothalamus of *db/db* Mice. Ten-week-old *db/db* mice were treated with vehicle (0.25% methylcellulose) or platensimycin at 10, 30, and 100 mpk (n = 5 at each dose per time point). Mice were dosed by mouth (p.o.) at 9:00 AM and food was removed. Tissues (liver and hypothalamus) were collected and quickly frozen at 1, 4, and 8 h postdose.

Chronic Treatment of Mice with Platensimycin. *db/db* or *db/+* mice were maintained on a standard chow diet or a high-fructose diet. For the *db/+* mouse study, 6-wk-old *db/+* mice were fed with a high-fructose diet (60 kcal%; D03012908; Research Diets). Platensimycin was added to drinking water (n = 8) to a final concentration of 3, 10, 30, and 100 mpk. One group was also fed a control diet (Diet 7012, Harlan). Three weeks after PTM treatment, mice were subjected to an insulin tolerance test using a dose of 0.75 U/kg of insulin. Body weight and food intake were measured for 4 wk before study termination. *db/db* mice (n = 7 mice per group) were treated starting at 7 wk of age; treatment lasted for 2 wk and consisted of a twice-daily oral gavage of platensimycin at 3 or 30 mpk, reconstituted in 0.25% methylcellulose. To determine 2-deoxy-D-glucose (2-DG) (Perkin-Elmer) uptake in muscle in vivo, *db/db* mice were dosed p.o. with rosiglitazone or PTM for 3 d. On the day of study, food was removed and mice were then dosed orally with compound 1 h before i.p. injection of [^{3}H]2-DG (1.5 μCi). Gastrocnemius muscle was collected at 60 min post-[^{3}H]2-DG dosing and processed.

Perfused Liver ^{13}C NMR Studies of Hepatic Glucose Production. The perfused liver method with ^{13}C NMR spectroscopy has been described in detail previously (1). Briefly, livers from anesthetized *db/db* mice treated with either PTM at 30 mpk or vehicle for 2–3 wk were harvested and perfused via a portal vein cannula with a Krebs–Henseleit buffer which was recirculated. Livers were then placed in a custom 20-mm NMR tube which was inserted into a 500-MHz wide-bore NMR spectrometer and the gluconeogenic substrate [2-^{13}C]pyruvate (7 mM) was added to the perfusate. ^{13}C NMR spectra (^{1}H-decoupled) were acquired from the liver/perfusate assembly at 11-min intervals, and the following NMR signals were monitored in real time: [1-^{13}C]glucose (96.8 ppm), [2-^{13}C]lactate (69.4 ppm), and [2-^{13}C]pyruvate (206 ppm). In separate calibration studies, known amounts of glucose, lactate, and pyruvate were added to the perfusion system and their NMR signals were used to convert the signals observed in the perfused

liver studies to absolute units in micromoles. For each study, the slopes of the time courses of pyruvate, lactate, and glucose were calculated to give the average fluxes of pyruvate consumption, lactate production, and glucose production, respectively.

1. Cohen SM (1991) In *Research in Perfused Liver*, eds Ballet F, Thurman RG (Libbey, London), pp 43–68.

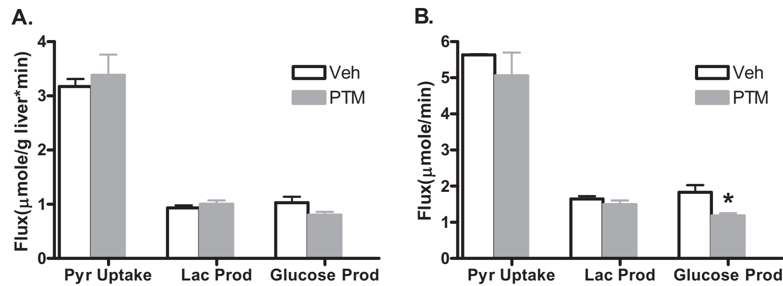

Fig. S1. Effects on pyruvate uptake (Pyr Uptake), lactate production (Lac Prod), and glucose production (Glucose Prod) in perfused *db/db* mouse liver following treatment with vehicle (Veh) or platensimycin (PTM) for 2 wk. *$P < 0.05$ versus vehicle ($n = 3$). Values shown are normalized for liver weight (*A*) or on a per-liver basis (*B*).

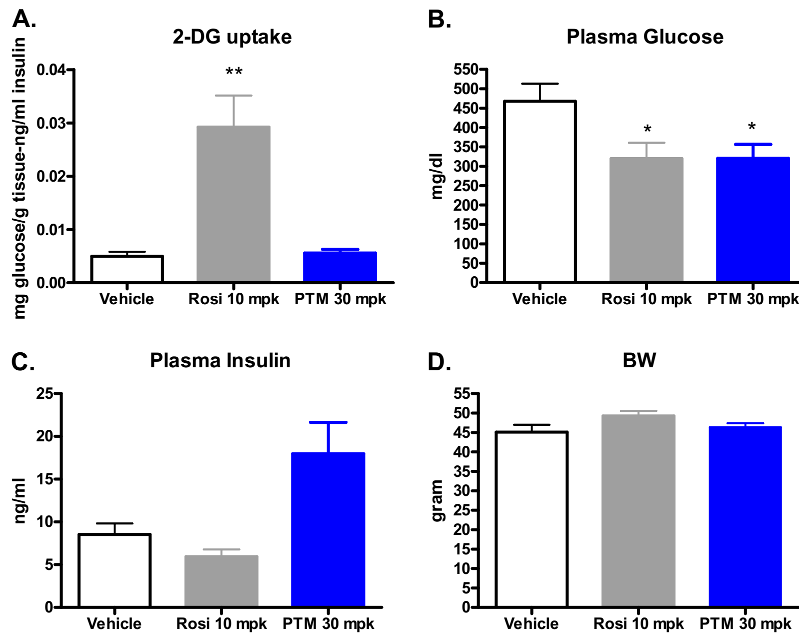

Fig. S2. Effect of PTM on 2-DG uptake in gastrocnemius muscle of *db/db* mice. *db/db* mice were dosed p.o. with vehicle, rosiglitazone (Rosi; 10 mpk), or platensimycin (PTM; 30 mpk) for 3 d. 2-DG uptake by gastrocnemius muscle was determined in *A*. Plasma levels of glucose and insulin are shown in *B* and *C*, and body weight (BW) is shown in *D*. $n = 5$. *$P < 0.05$, **$P < 0.01$ versus vehicle control.