cm)). Their body weights were measured at approximately 10:00 A.M. every day for 7 days before dosing. Mice were administered orally with a solution of compounds at a dose of 30 mg/kg or with the vehicle (1% ethanol and 0.5% carboxymethyl cellulose (CMC) in distilled water) at a volume of 10 mL/kg of animal at approximately 10:00 A.M. every day for 7 days. At the final day of dosing, animals were fasted from 17:00 P.M. and given water *ad libitum*. On the next day at approximately 10:00 A.M., animals were weighed and anesthetized with diethyl ether. Blood and liver were removed immediately, and the liver weight was measured. Approximately 1 mL of blood in an Eppendorf sample tube was centrifuged to afford the serum sample. Each blood sample was centrifuged at 9,000 rpm for 5 min at r.t..

4.4. Observation of side effects after once-daily oral administration of 30 mg/kg for 28 consecutive days in SD rats

Five-week-old male and female SD rats were purchased from Charles River Laboratories Japan, Inc.. This experiment was conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center, and all procedures were approved by the Animal Research Control Committee of Okayama University. After the arrival of the rats, all were group-housed and acclimated to the colony for 1 day before the experiment. Before the experiment, they were housed with three rats per cage, with free access to water and chow pellets in a light (12 hr on, 8:00 AM /12 hr off, 8:00 PM), temperature (23 ± 1°C), and relative humidity (50 ± 3%)-controlled environment. At 1 day before experiments, rats were assigned to experimental groups to minimize the variance between groups based on the measured weight (three per cage (34.5 x 40.3 x 17.7 cm)). Their body weights were measured at approximately 10:00 A.M. every day for 28 days before dosing. Rats were administered orally with a solution of compounds at a dose of 30 mg/kg or with the vehicle (1% ethanol and 0.5% carboxymethyl cellulose (CMC) in distilled water) at a volume of 5 mL/kg of animal at approximately 10:00 A.M. every day for 28 days. At the final day of dosing, animals were fasted from 17:00 P.M. and given water *ad libitum*. On the next day at approximately 10:00 A.M., animals were weighed and anesthetized with diethyl ether (maintained with isoflurane). Blood, liver, brain, kidney, spleen, and testis (male only) were removed immediately. The liver, brain, kidney, spleen, and testis were weighed and frozen with liquid nitrogen. Approximately 10 mL of blood in a centrifuge tube was centrifuged at 2,000*g* for 10 min at 4°C to obtain a serum sample.

4.5. Evaluation of blood glucose-lowering activities in KK-A^y mice

Type 2 diabetic KK-A^y mice, in which the A^y allele at the agouti locus was transferred to the inbred KK strain by repetitive back-crossing, were used as the congenic strain. The introduction of the A^y allele caused DM and massive hereditary obesity. The KK-A^y mice (CLEA Japan Inc., Tokyo, Japan) were allowed free access to solid food and tap water. This experiment was conducted in

accordance with the Guidelines for Animal Experiments at Kyoto Pharmaceutical University (KPU), and all procedures were approved by the Experimental Animal Research Committee at KPU. The animals were housed in an air-conditioned room at a temperature of 23 ± 1 °C and a humidity of 60 ± 10%, with lights on from 8:00 AM to 20:00 PM. After the determination of their blood glucose levels, the mice received daily oral administrations of compounds dissolved in polyethylene glycol 400 (PEG) at approximately 10:00 AM for 14 days.

4.6. Measurements of blood parameters

White blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets (PLT) were measured by using a pocH-100i (Sysmex). Alanine aminotransferase (AST), aspartate aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), creatinine (CRE), blood urea nitrogen (BUN), triglyceride (TG), total cholesterol (TCHO), and glucose (GLU) were measured by using a Fuji Dry Chem system (Dry Chem 4000V, Fuji Medical Co., Tokyo, Japan).

4.7. Measurements of metabolic parameters

Blood samples for analysis of glucose level were obtained from the tail vein of the mice and serum glucose levels were measured using Glucocard. Serum concentrations of total choresterol (TCHO) and triglyceride (TG) were determined by a Fuji Dry Chem system (Fuji Medical Co., Tokyo, Japan) as described above. The level of hemoglobin A1C (HbA1C) was measured 24 hr after the final administration of compounds by an immunoassay method (DCA2000). Serum levels of insulin were determined with Glazyme insulin-EIA test (Wako Pure Chemicals Co., Osaka, Japan). Serum levels of adiponectin were determined with Adiponectin immunoassay kit (R&D Systems Inc., Minneapolis, MN, USA).

4.8. RNA Preparation and quantitative real-time RT-PCR

Fifty milligrams of liver tissue from KK-A^{y} mice described above was resected and mechanically homogenized with a Politron PT 10/35 (Kinematica Inc., Littau-Luzern, Switzerland) in 0.5 mL of Trizol reagent (Invitrogen, Carlsbad, CA). Total RNA was extracted as previously described (*S4*). Quantitative real-time RT-PCR analysis was performed using a LightCycler rapid thermal cycler system (Roche Applied Science, Mannheim, Germany) following the protocol previously reported (*S5*). Two micrograms of total RNA was reverse-transcribed by random hexamer priming using ReverTra Ace (Toyobo, Osaka, Japan). The PCR mixture consisted of 1x SYBR Green PCR Master Mix (Toyobo), which includes DNA polymerase, SYBR Green I Dye, dNTPs, PCR buffer, 10 pmol

forward and reverse primers and cDNA of samples in a total volume of 20 μL. The amplification of a housekeeping gene, *Rps18*, was used to normalize the efficiency of cDNA synthesis and the amount of RNA applied. PCR was performed with initial denaturation at 94°C for 30 sec, followed by amplification for 40 cycles, each cycle consisting of denaturation at 95°C for 5 sec, annealing at 60°C for 15 sec, and polymerization at 72°C for 15 sec. Primers used for this study are listed in Table S5.

4.9. Supplementary data 2

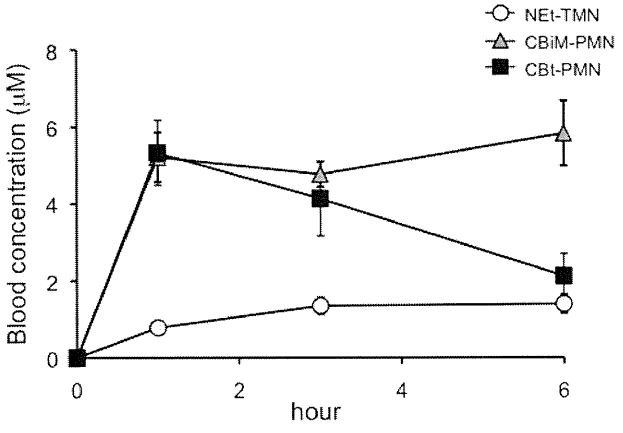

Figure S4. Plasma concentrations of NEt-TMN (**5**), CBiM-PMN (**11a**) and CBt-PMN (**11b**) in mice after single oral administration of 30 mg/kg. Circles, triangles, and squares indicate NEt-TMN (**5**), CBiM-PMN (**11a**), and CBt-PMN (**11b**), respectively. The data (n = 5–9) represent the mean ± s.e.m. Significant differences: * $p<0.05$ vs. vehicle. ** $p<0.01$ vs. vehicle.

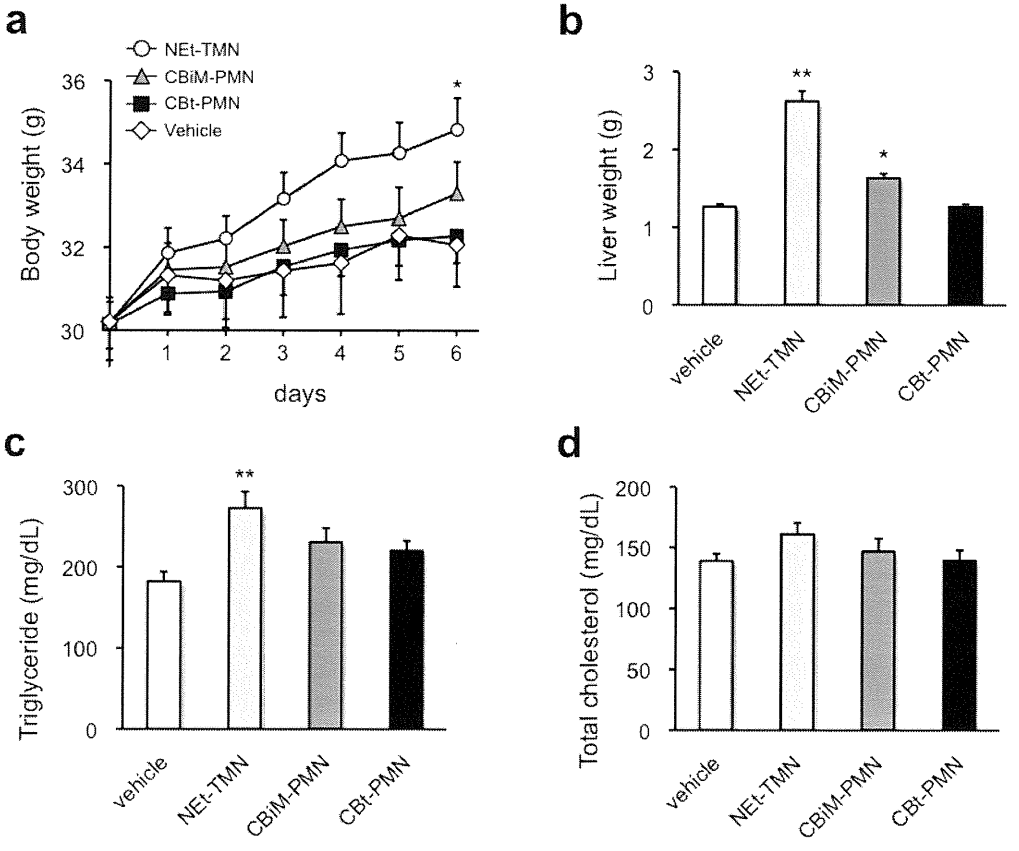

Figure S5. Evaluation of adverse effects of repeated oral administration of compounds at 30 mg/kg/day to male ICR mice for 7 consecutive days. **a**) Time course of body weight. Circles, triangles, and squares indicate NEt-TMN (**5**), CBiM-PMN (**11a**), and CBt-PMN (**11b**), respectively. **b-d**) Effects of compounds on liver weight gain, serum triglyceride and total cholesterol, respectively. The data (n = 7–23) represent the mean ± s.e.m. Significant differences: * $p<0.05$ vs. vehicle. ** $p<0.01$ vs. vehicle.

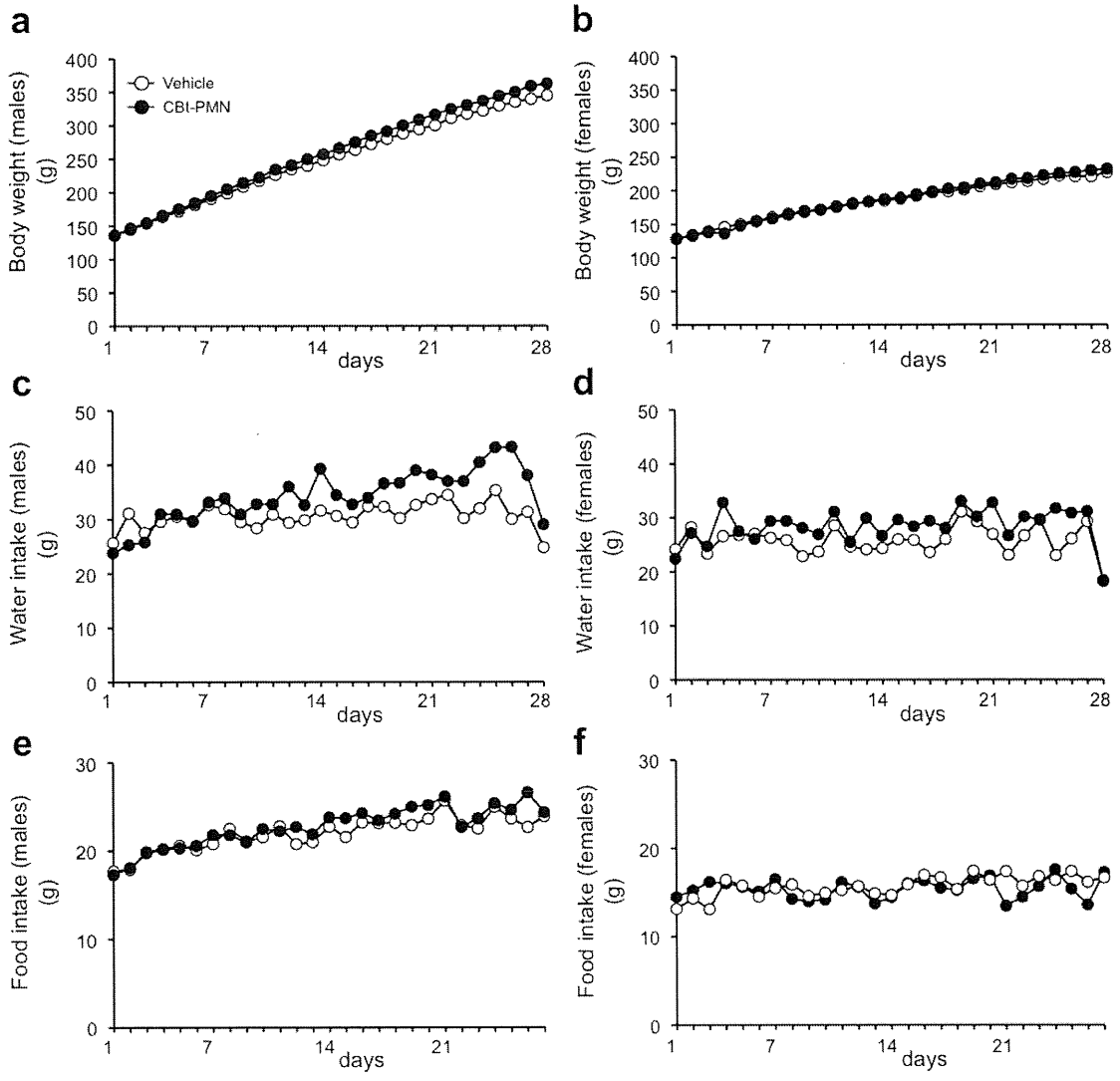

Figure S6. Body weight gain, water intake change, and food intake change of male or female SD rats treated with oral administration of vehicle or CBt-PMN (**11b**) at 30 mg/kg/day for 28 consecutive days. **a**–**b**) Body weight gain. **c**–**d**) Water intake change. **e**–**f**) Food intake change. Males: **a**, **c**, and **e**. Females: **b**, **d**, and **f**. Open and closed circles indicate vehicle and CBt-PMN (**11b**) treatment, respectively. n = 6.

Table S2. Serum parameters of male ICR mice after oral administration of vehicle or CBt-PMN (**11b**) at 30 mg/kg/day for 7 consecutive days (n = 7–8)

	Vehicle	NEt-TMN (**5**)	CBiM-PMN (**11a**)	CBt-PMN (**11b**)
AST (U/l)	50.5 ± 5.6	87.9 ± 18.9 *	51.3 ± 3.9	48.9 ± 3.0
ALT (U/l)	21.4 ± 1.5	43.5 ± 6.0 **	32.7 ± 6.4	21.9 ± 0.7
γ-GTP (U/l)	7.9 ± 0.8	7.1 ± 0.3	6.4 ± 0.3	6.5 ± 0.2
ALP (U/l)	305.1 ± 27.1	968.9 ± 115.0 **	456.0 ± 51.5	391.9 ± 37.5
CRE (mg/dL)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
BUN (mg/dL)	25.1 ± 1.4	26.0 ± 1.8	24.1 ± 2.1	25.1 ± 1.9
GLU (mg/dL)	107.5 ± 9.8	177.8 ± 8.8 **	125.7 ± 7.2	95.6 ± 4.2

AST : aspartate aminotransferase, ALT : alanine aminotransferase, γ-GTP : γ-glutamyl transpeptidase, ALP : alkaline phosphatase, CRE : creatinine, BUN : blood urea nitrogen, GLU : glucose.

Data are mean ± s.e.m.; Significant differences: * $p<0.05$ vs. vehicle. ** $p<0.01$ vs. vehicle.

Table S3. Organ weights of male or female SD rats after oral administration of vehicle or CBt-PMN (**11b**) at 30 mg/kg/day for 28 consecutive days (n = 6)

	Males		Females	
	Vehicle	CBt-PMN (**11b**)	Vehicle	CBt-PMN (**11b**)
Weight (g)	324.3 ± 8.7	337.8 ± 4.6	213.4 ± 2.1	216.1 ± 10.7
Brain (g)	2.0 ± 0.1	2.0 ± 0.1	1.8 ± 0.0	1.7 ± 0.0
Liver (g)	9.7 ± 0.6	9.7 ± 0.4	5.9 ± 0.2	6.2 ± 0.4
Kidney (g)	2.5 ± 0.1	2.6 ± 0.1	1.6 ± 0.0	1.5 ± 0.1
Spleen (g)	0.6 ± 0.0	0.7 ± 0.1	0.4 ± 0.0	0.5 ± 0.0
Testis (g)	3.9 ± 0.2	4.5 ± 0.1 *		

Data are mean ± s.e.m.; Significant differences: * $p<0.05$ vs. vehicle.

Table S4. Hematological and serum parameters of male and female SD rats after oral administration of vehicle or CBt-PMN (**11b**) at 30 mg/kg/day for 28 consecutive days (n = 6)

	Males			Females		
	vehicle	CBt-PMN	Reference[c]	vehicle	CBt-PMN	Reference[c]
WBC ($\times 10^2$/mL)[a]	71.7 ± 4.2	92.5 ± 18.6	–	76.5 ± 9.3	72.5 ± 7.5	–
RBC ($\times 10^4$/mL)[a]	741.8 ± 17.4	746.2 ± 11.7	–	778.3 ± 18.1	767.3 ± 12.0	–
PLT ($\times 10^4$/mL)[a]	123.3 ± 5.7	139.9 ± 13.1	–	129.3 ± 4.9	140.3 ± 7.1	–
HGB (g/dL)[a]	14.8 ± 0.2	14.4 ± 0.2	14.4 – 16.0	14.7 ± 0.2	14.5 ± 0.2	13.7 – 15.7
HCT (%)[a]	45.5 ± 0.6	45.0 ± 0.7	41.2 – 47.3	45.7 ± 0.8	44.8 ± 0.6	9.6 – 46.0
MCV (fL)[a]	61.4 ± 0.8	60.4 ± 0.4	53.0 – 59.5	58.7 ± 0.6	58.4 ± 0.6	53.6 – 58.1
MCH (pg)[a]	20.0 ± 0.2	19.3 ± 0.2 *	18.3 – 20.0	18.9 ± 0.3	18.9 ± 0.2	18.6 – 20.0
MCHC (g/dL)[a]	32.5 ± 0.1	31.9 ± 0.2 *	32.7 – 35.7	32.2 ± 0.3	32.2 ± 0.1	32.8 – 36.2
AST (U/l)[b]	65.7 ± 3.5	75.2 ± 2.8 *	87.0 – 114.0	60.2 ± 1.6	64.8 ± 2.0 *	85.0 – 123.0
ALT (U/l)[b]	27.8 ± 1.4	37.7 ± 2.0 **	28.0 – 40.0	21.5 ± 2.1	29.3 ± 1.9 **	25.0 – 36.0
γ-GTP (U/l)[b]	8.2 ± 0.4	8.3 ± 0.2	0.0 – 1.0	8.3 ± 0.2	8.7 ± 0.3	0.0 – 0.4
ALP (U/l)[b]	585.7 ± 34.3	607.5 ± 39.7	–	434.3 ± 50.7	476.5 ± 75.2	–
CRE (mg/dL)[b]	0.2 ± 0.0	0.2 ± 0.0	0.5 – 0.6	0.3 ± 0.0	0.3 ± 0.0	0.5 – 0.6
BUN (mg/dL)[b]	16.4 ± 0.7	11.8 ± 0.4 **	13.0 – 16.0	13.8 ± 0.7	14.5 ± 0.9	11.0 – 16.0
TG (mg/dL)[b]	44.8 ± 2.8	50.8 ± 2.9	61.0 – 99.0	55.0 ± 4.9	77.5 ± 5.5 **	42.0 – 74.0
TCHO (mg/dL)[b]	56.2 ± 7.5	34.0 ± 4.5 *	54.0 – 74.0	25.7 ± 3.7	44.8 ± 10.6	67.0 – 87.0
GLU (mg/dL)[b]	181.2 ± 16.1	149.8 ± 17.7	112.0 – 176.0	118.3 ± 5.7	145.5 ± 10.6 *	113.0 – 185.0

a. Measured with a pocH-100i (Sysmex).

b. Measured with a Fuji Dry Chem 4000V (Fuji Medical Co., Tokyo, Japan).

c. These data were cited from the Clinical Laboratory Parameters for Crl:CD(SD) Rats (CRL_Mar,2006) by Charles River®.

Data are mean ± s.e.m.; Significant differences: * $p<0.05$ vs. vehicle. ** $p<0.01$ vs. vehicle.

Table S5. Primer list

Primer		Sequence
Irs1	Forward	5'-CAAGACGCTCCAGTGAGGATTTAAG-3'
	Reverse	5'-AGACGTGAGGTCCTGGTTGTGA-3'
Irs2	Forward	5'-TGGTTCTCACAAGAGTTCCAGCA-3'
	Reverse	5'- AGCTATTGGGACCACCACTCCTAA-3'
Slc2a1	Forward	5'-TGTGGGCATGTGCTTCCAGTA-3'
	Reverse	5'-GCCTTTGGTCTCAGGGACTTTG-3'
Slc2a2	Forward	5'-GGCATCAGCCAGCCTGTGTA-3'
	Reverse	5'-CATGCCAATCATCCCGGTTAG-3'
G6Pc	Forward	5'-CAGCAACAGCTCCGTGCCTA-3'
	Reverse	5'-TCCCAACCACAAGATGACGTTC-3'
Pck	Forward	5'-TCTTTGGTGGCCGTAGACCTG-3'
	Reverse	5'-GCCAGGTATTTGCCGAAGTTGTAG-3'
Gck	Forward	5'-CGGTGAGCTGGACGAGTTC-3'
	Reverse	5'-ACCAGCTCGCCCATGTACTT-3'
Scd1	Forward	5'-TCCGGAACCGAAGTCCAC-3'
	Reverse	5'-GTGGTCGTGTAAGAACTGGAGATCT-3'
Fasn	Forward	5'-ATTGGCTCCACCAAATCCAAC-3'
	Reverse	5'-CCCATGCTCCAGGGATAACAG-3'
Srebp1c	Forward	5'-GGTTTTGAACGACATCGAAGA-3'
	Reverse	5'-CGGGAAGTCACTGTCTTGGT-3'

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