# 4-(*trans*-4-Methylcyclohexyl)-4-Oxobutyric Acid (JTT-608). A New Class of Antidiabetic Agent

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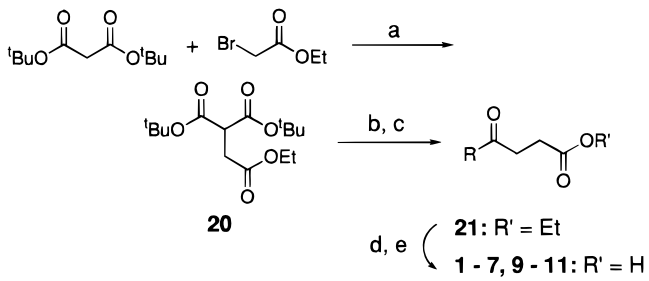
During an investigation of drugs for improving the $\beta$-cell response to glucose, we found that 4-cyclohexyl-4-oxobutyric acid selectively improved glucose-stimulated insulin release and glucose tolerance in both normal and diabetic rats. A series of 4-cycloalkyl-4-oxobutyric acids and related compounds were synthesized and evaluated for their effects on the glucose tolerance test and fasting euglycemia. This study elucidated the structural requirements for drug activity and determined that the optimum compound was 4-(*trans*-4-methylcyclohexyl)-4-oxobutyric acid **7** (JTT-608). This compound improved glucose tolerance from an oral dose of 3 mg/kg and did not change fasting euglycemia even at an oral dose of 30 mg/kg. Selective improvement of glucose-induced insulin secretion was observed in studies using neonatal streptozotocin rats (nSTZ rats) and perfused pancreases isolated from nSTZ rats.

## Introduction

Type 2 diabetes is a metabolic disorder characterized by hyperglycemia, which leads to chronic complications such as neuropathy, nephropathy, retinopathy, and premature atherosclerosis.[1] Maintaining good glycemic control is important to prevent diabetic complications.[2] The pathogenesis of type 2 diabetes is heterogeneous, and hyperglycemia in these patients is caused by defects in the secretion and/or action of insulin.[3] Accordingly, both improvement of defective insulin secretion by pancreatic $\beta$-cells and a reduction in the insulin resistance of peripheral tissues are desirable for achieving tight blood glucose control. Many drugs from the glitazone family of insulin sensitizers have been developed to treat the type 2 diabetic patients with insulin resistance,[4] and the thazolidinedione-based agent troglitazone has been approved for use in Japan, the United Kingdom, and the United States. On the other hand, sulfonylureas are used to treat defective insulin secretion in type 2 diabetes. The insulin-releasing activity of sulfonylureas is considered to be mediated by inhibition of the ATP-sensitive $K^+$ channel in pancreatic $\beta$-cells, and their hypoglycemic activity is dose-dependent.[5] Therefore, the risk of hypoglycemia is always present when using sulfonylureas.[5] The $\beta$-cell response to glucose and the first phase of glucose-induced insulin secretion are characteristically reduced in type 2 diabetes.[6] Accordingly, an ideal drug for correcting the abnormalities of insulin secretion in this disease would restore the normal $\beta$-cell response to glucose, but such a drug does not exist yet.[7] We have attempted to develop a drug that can improve glucose tolerance by restoring $\beta$-cell sensitivity to glucose and insulin secretion. During screening studies, we found that 4-cyclohexyl-4-oxobutyric acid **1** improved glucose-induced insulin release and glucose tolerance at an oral dose of 30 mg/kg while not altering fasting euglycemia at the same dose. Structure−activity and optimization studies on a series of 4-cycloalkyl-4-oxobutyric acids and related compounds disclosed the structural requirements for this action and eventually led to the optimum compound, 4-(*trans*-4-methylcyclohexyl)-4-oxobutyric acid **7** (JTT-608). This compound improved glucose tolerance from an oral dose of 3 mg/kg and did not alter fasting euglycemia at an oral dose of 30 mg/kg in normal and diabetic neonatal streptozotocin rats (nSTZ rats). Selective improvement of glucose-induced insulin secretion was observed in studies using nSTZ rats and perfused pancreases isolated from nSTZ rats. Since the pharmacological properties of compound **7** suggested that it might improve glycemic control in type 2 diabetes, it is currently undergoing evaluation in clinical trials.

**Scheme 1**[a]




[a] Reagents: (a) NaH; (b) NaH, R-COCl; (c) $TsOH \cdot H_2O$, toluene, reflux; (d) NaOH; (e) recrystallization.

## Chemistry

Scheme 1 shows the preparation of 4-oxobutyric acids. The triester **20** was prepared by alkylation of di-*tert*-butyl malonate with ethyl bromoacetate. Acylation of **20** with the corresponding acid chlorides and subsequent decarboxylation gave the ethyl 4-oxobutyrates **21**. Basic hydrolysis of **21**, followed by crystallization, provided the 4-oxobutyric acids (**1**−**7**, and **9**−**11**). The pure trans isomers (**7** and **10**−**11**) were obtained by repeated crystallization because the 4-alkylcyclohexyl compounds were easily isomerized to mixtures of the trans and cis isomers at a 9:1 ratio under both basic and acidic conditions.

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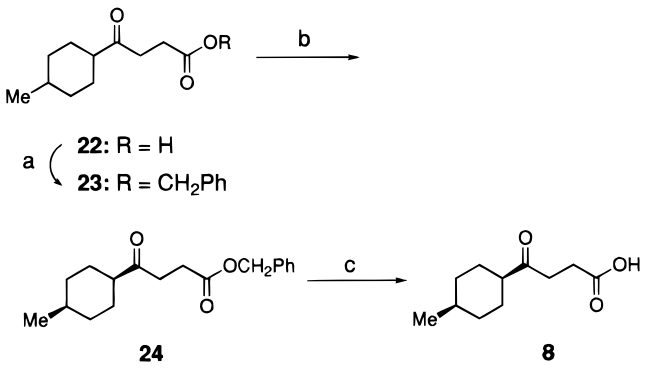
**Scheme 2**[a]




[a] Reagents: (a) $NaHCO_3$, $PhCH_2Br$; (b) HPLC; (c) $H_2$, Pd-C.

Preparation of compound **8** (the cis isomer of compound **7**) is shown in Scheme 2. The benzyl ester **23** (a mixture of cis and trans isomers at a 1:1 ratio) was synthesized from **22** (also a mixture of cis and trans isomers at a 1:1 ratio), which was obtained from the supernatant after separating trans isomer **7** by crystallization. The cis isomer **24** was isolated from **23** by high-performance liquid chromatography (HPLC) on a YMC SIL-5-B-06 (30 mm × 250 mm i.d.) column. Reductive cleavage of the benzyl ester of **24** gave the cis isomer **8**.

Decarbonyl compound **12** was prepared from compound **7** by Wolff−Kishner reduction.

Scheme 3 shows preparation of the sulfoxide **13** and sulfone **14**. The sulfide **25** was prepared by reaction of *cis*-4-methylcyclohexyl *p*-toluenesulfonate[8] with ethyl 3-mercaptopropionate in the presence of cesium carbonate. Oxidation of **25** with 1 equiv of *m*-chloroperoxybenzoic acid, followed by basic hydrolysis, gave the sulfoxide **13**. On the other hand, oxidation of **25** with 2 equiv of *m*-chloroperoxybenzoic acid, followed by acidic hydrolysis, provided the sulfone **14**.

Esterification of *trans*-4-methylcyclohexanecarboxylic acid **26** with *tert*-butyl bromoacetate, followed by acidic hydrolysis, gave the ester **15**. Coupling of **26** with glycine benzyl ester, followed by reductive cleavage of the benzyl ester group, yielded the amide **16**. Methylation of **26** with methyllithium and subsequent bromination with bromine afforded the α-bromoketone **27**. Alkylation of **27** with benzyl acetoacetate followed by reductive cleavage of the benzyl ester and subsequent decarboxylation furnished the 1,4-diketone **17** via **28** (Scheme 4).

Preparation of 5-oxovaleric acid **19** is shown in Scheme 5. The 3-oxoester **29** was synthesized by reaction of 4-methylcyclohexyl carboxyl chloride and the dianion of ethyl hydrogen malonate (prepared with 2 equiv of butyllithium), followed by decarboxylation.[9] The intermediate **30** was obtained by alkylation of **29** with ethyl 3-bromopropionate using potassium *tert*-butoxide as the base. Basic hydrolysis of **30** and subsequent decarboxylation, followed by recrystallization, gave 5-oxovaleric acid **19**.

## Results and Discussion

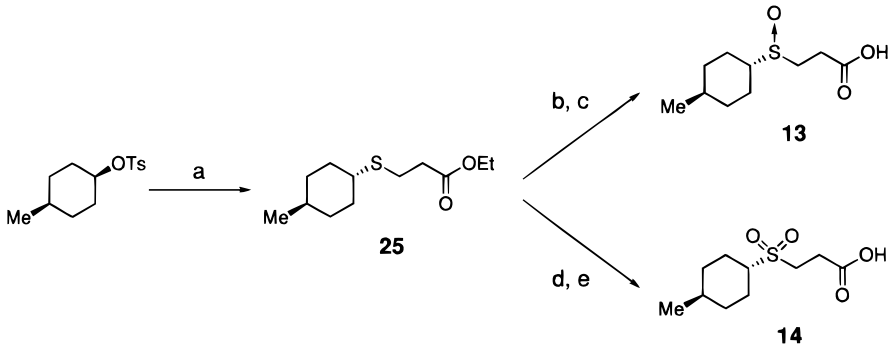
The effects of the synthesized compounds on glucose tolerance and fasting euglycemia in normal rats are summarized in Table 1.

First, the cyclohexyl moiety of compound **1** was varied in order to investigate its influence on the pharmacological activity. Since the cyclopentyl compound **2** and the cycloheptyl compound **3** showed no activity, we examined the effects of alkyl substituents on the cyclohexyl ring of compound **1**. Although introduction of a methyl group at the 1-, 2-, or 3-position of the cyclohexyl ring (**4**, **5**, and **6**) decreased or extinguished activity, *trans*-4-methylcyclohexyl compound **7** was found to be active from an oral dose of 3 mg/kg. Introduction of another methyl group at the axial 4-position of compound **7** (4,4-dimethyl compound **9**) led to reduction of activity. Accordingly, an equatorial methyl substituent at the *trans*-4-position of the cyclohexylcarbonyl moiety was recognized to specifically enhance activity, while a methyl substituent at the 1-, 2-, 3-, or cis 4-position of the cyclohexylcarbonyl moiety decreased the activity. Next, the effect of replacing the *trans*-4-methyl group of compound **7** with a larger ethyl or propyl group was examined. However, both *trans*-4-ethylcyclohexyl compound **10** and *trans*-4-*n*-propylcyclohexyl compound **11** showed no activity. A larger alkyl substituent than the methyl group strongly inhibited activity, possibly as a result of its hindrance. These results demonstrated that the *trans*-4-methylcyclohexyl structure achieved the optimum activity. However, the *cis*-4-methylcyclohexyl compound **8** also exhibited activity, despite the great difference in steric structure between cis isomer **8** and trans isomer **7**. NMR showed that the coupling constant values of the proton signal at the 1-position on the cyclohexane ring of compound **7** were 3.3 and 12.7 Hz, while the values for compound **8** were 4.6 and 6.4 Hz. These results indicated that H1 of compound **7** was axial and H1 of compound **8** was equatorial (Karplus−Conroy equation[10]), so that the steric structures of these cis and trans isomers were greatly different. The cis compound **8** was easily isomerized to the more stable trans 1,4-diequatorial compound **7** under mildly basic conditions. Therefore, the residual activity of compound **8** may have been attributable to partial isomerization to the trans form **7** under the experimental (physiological) conditions.

Subsequently, the influence of the γ-ketocarboxylic acid moiety of compound **7** on the pharmacological activity was investigated. Deletion of the oxygen atom from the ketone structure (**12**) caused loss of activity, indicating that the ketone group at this site was important. Then the effect of replacing the ketone moiety of compound **7** with ketone-bioisosters (sulfoxide **13**, sulfone **14**, ester **15**, and amide **16**) was examined, but none of them showed any activity. These results confirmed the importance of the ketone group. To determine the necessity of the carboxylic acid group for pharmacological activity, we altered it to a ketone (**17**) or an amide (**18**) or increased the number of methylene units between the ketone group and the carboxylic acid group (**19**). The δ-ketocarboxylic acid **19** showed loss of activity, indicating the necessity of a carboxylic acid group located three C−C bonds distant from the central ketone. The ketone **17** and the amide **18** showed less activity when compared to compound **7**, but the residual activity of compounds **17** and **18** indicated the importance of the carbonyl structure in the carboxylic acid group.

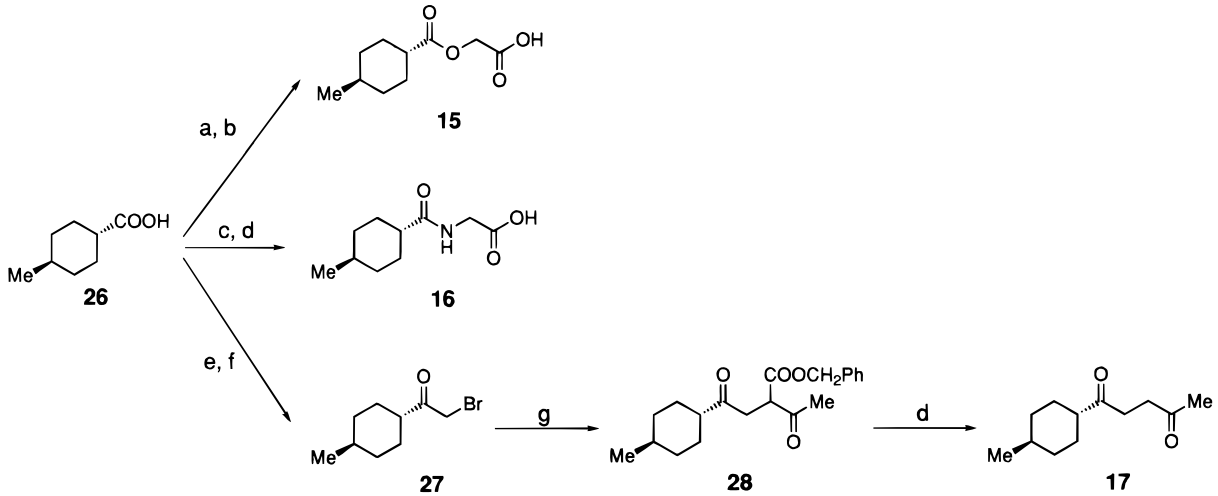
When the compounds showing activity in the glucose tolerance test (compounds **1**, **7**, **8**, **17**, and **18**) were

**Scheme 3**[a]



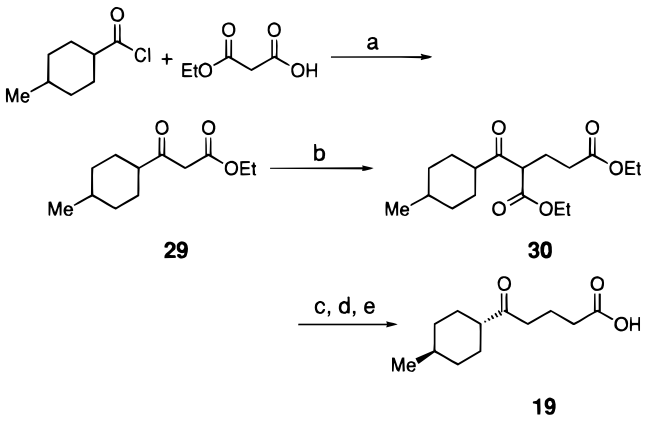

[a] Reagents: (a) $Cs_2CO_3$, ethyl 3-mercaptopropionate; (b) 1 equiv of *m*-chloroperoxybenzoic acid; (c) LiOH; (d) 2 equiv of *m*-chloroperoxybenzoic acid; (f) conc HCl.

**Scheme 4**[a]




[a] Reagents: (a) $K_2CO_3$, *tert*-butyl bromoacetate; (b) TFA; (c) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1-hydroxybenzotriazole, glycine benzyl ester; (d) $H_2$, Pd-C; (e) MeLi; (f) $Br_2$; (g) NaH, benzyl acetoacetate.

**Scheme 5**[a]



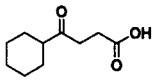
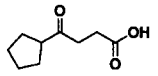
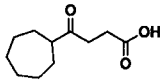
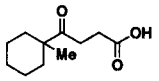
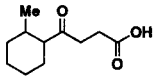
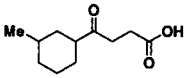
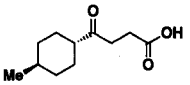
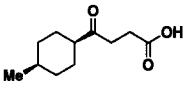
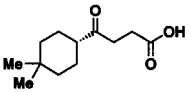
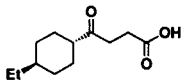
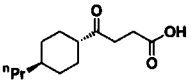
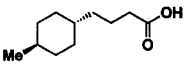
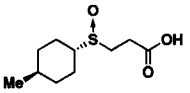
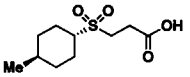
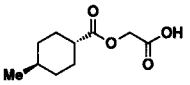
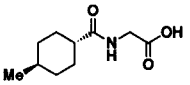
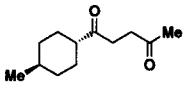
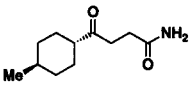
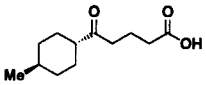

[a] Reagents: (a) $^{n}$BuLi; (b) $^{t}$BuOK, ethyl 3-bromopropionate; (c) NaOH; (d) 100 °C; (e) recrystallization.

administered to fasting normal rats, there was no influence on fasting euglycemia at an oral dose of 30 mg/kg. Accordingly, the effect of these compounds was dependent on the glucose level, and there was a selective response to a high blood glucose level. This characteristic was considered to be favorable for achieving glucose homeostasis without causing hypoglyceia in patients with type 2 diabetes. We then tested compound **7**, which was optimum in normal rats, to determine its effect in diabetic rats.

Figure 1 shows the effect of compound **7** on glucose tolerance and fasting euglycemia in diabetic rats. We used neonatal streptozotocin rats (nSTZ rats) as a model of diabetes because these rats have a low insulin response to glucose and show postprandial hyperglycemia as well as an oral glucose tolerance in the diabetic range.[11] nSTZ rats were created by giving a single subcutaneous injection of 120 mg/kg of streptozotocin to neonatal (1.5 days old) male Sprague−Dawley rats.[12] Compound **7** did not change fasting euglycemia at oral doses of 10 and 30 mg/kg, but slightly decreased it at 100 mg/kg. On the other hand, the compound strongly decreased hyperglycemia induced by an oral glucose load and dose-dependently increased glucose-induced insulin secretion at oral doses of 10, 30, and 100 mg/kg. We thus confirmed that compound **7** not only enhanced glucose tolerance in normal rats but also improved glucose intolerance in diabetic rats.

Figure 2 shows the effect of 100 $\mu$M tolbutamide and 200 $\mu$M compound **7** (similar to the blood level of JTT-608 achieved by an oral dose of 30 mg/kg in rats) on insulin release by perfused pancreases isolated from normal rats and stimulated with 2.8 and 7.0 mM glucose. Tolbutamide promoted a marked increase of insulin secretion at 2.8 mM glucose, and only enhanced the second phase of insulin secretion in response to 7.0 mM glucose. On the other hand, compound **7** induced little insulin secretion at the low glucose concentration

**Table 1.** Effect on Glucose Tolerance and Euglycemia in Normal Rats

| | | effect on glucose tolerance (%)[a] | | | | | |
|---|---|---|---|---|---|---|---|
| compd | structure | 100 mg/kg | 30 mg/kg | 10 mg/kg | 3 mg/kg | 1 mg/kg | effect on fasting euglycemia[b] |
| 1 |  | 26 | 27 | inactive | | | inactive |
| 2 |  | inactive | | | | | |
| 3 |  | inactive | | | | | |
| 4 |  | 28 | inactive | | | | |
| 5 |  | inactive | | | | | |
| 6 |  | 37 | inactive | | | | |
| 7 |  | 65 | 41 | 32 | 24 | inactive | inactive |
| 8 |  | 44 | 32 | inactive | | | inactive |
| 9 |  | 34 | inactive | | | | |
| 10 |  | inactive | | | | | |
| 11 |  | inactive | | | | | |
| 12 |  | inactive | | | | | |
| 13 |  | inactive | | | | | |
| 14 |  | inactive | | | | | |
| 15 |  | inactive | | | | | |
| 16 |  | inactive | | | | | |
| 17 |  | 31 | 30 | inactive | | | inactive |
| 18 |  | 47 | 27 | inactive | | | inactive |
| 19 |  | inactive | | | | | |

[a] Antihyperglycemic activity in glucose-loaded (1 g/kg ip) rats at doses of 3, 10, and 30 mg/kg po. Values show the percent decrease of hyperglycemia at 30 min after glucose loading relative to the vehicle control. [b] Hypoglycemic activity in fasting rats at a dose of 30 mg/kg po.

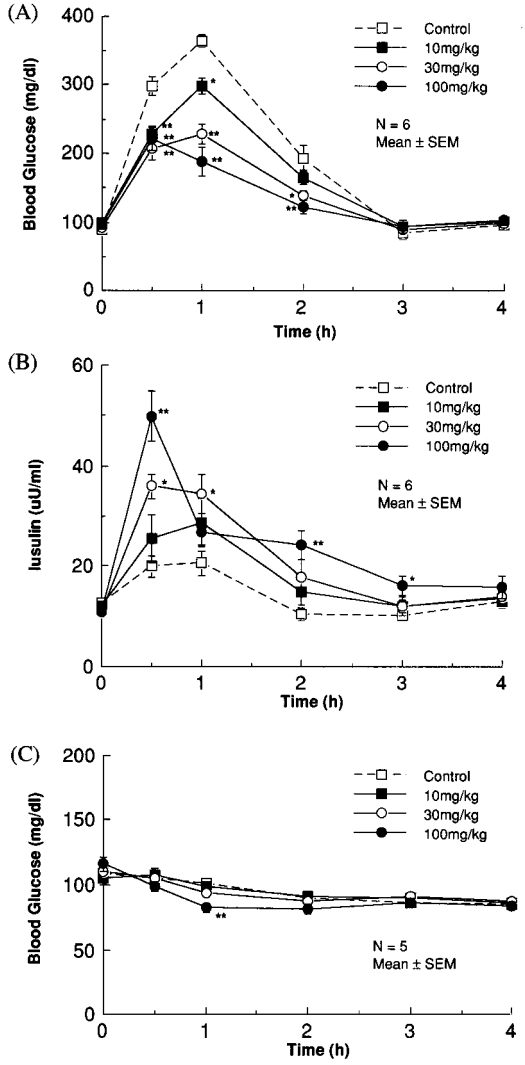



**Figure 1.** Effect of compound **7** (JTT-608) on glucose tolerance and fasting euglycemia in diabetic nSTZ rats. (A) Time course of blood glucose and (B) insulin after glucose loading (2 g/kg, po). Compound **7** (10, 30, or 100 mg/kg) or vehicle (control) was administered orally at 10 min before glucose loading. (C) Time course of blood glucose after oral administration of compound **7** (10, 30, or 100 mg/kg) or vehicle (control) without glucose loading. $*p < 0.05$, $**p < 0.01$; significantly different from the control by Dunnett's two-tailed test.

(2.8 mM), but it enhanced both the first and second phase of insulin secretion induced by 7.0 mM glucose. These results indicated that the effect of compound **7** was dependent on the glucose level and was different from that of tolbutamide.

Figure 3 shows the effect of 200 $\mu$M compound **7** on insulin release by perfused pancreases isolated from diabetic nSTZ rats and stimulated with 2.8 and 11.1 mM glucose. Compound **7** induced little insulin secretion at a low glucose concentration (2.8 mM), but it improved both the first and second phase of insulin secretion in response to 11.1 mM glucose. Thus, we confirmed that compound **7** not only selectively enhanced glucose-induced insulin secretion by the normal pancreas but also selectively improved the impaired insulin secretory response to glucose of the diabetic pancreas. The changes of insulin secretion by perfused pancreases indicated that compound **7** acted directly on the pancreas and enhanced the $\beta$-cell response to glucose.

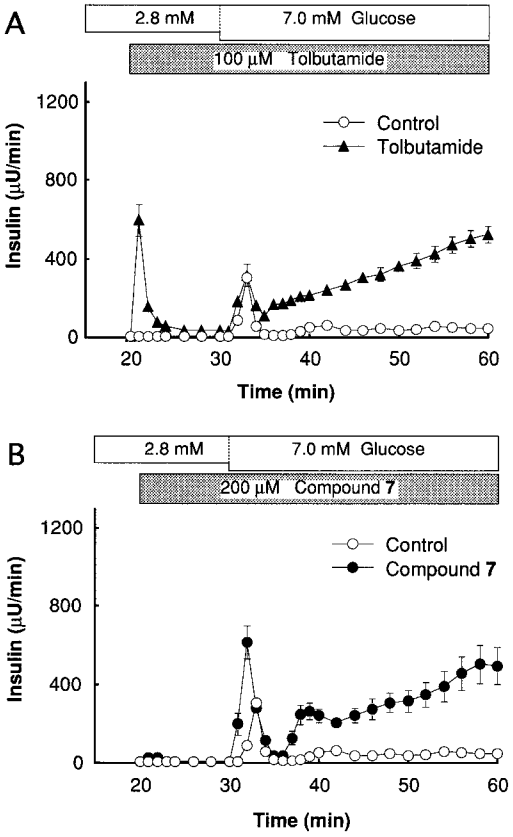



**Figure 2.** (A) Effects of 100 $\mu$M tolbutamide and (B) 200 $\mu$M compound **7** on insulin secretory response to 2.8 mM glucose or 7.0 mM glucose of perfused pancreases isolated from normal Wistar rats (7 weeks old). Values are the mean ± SE. ($N = 8$).

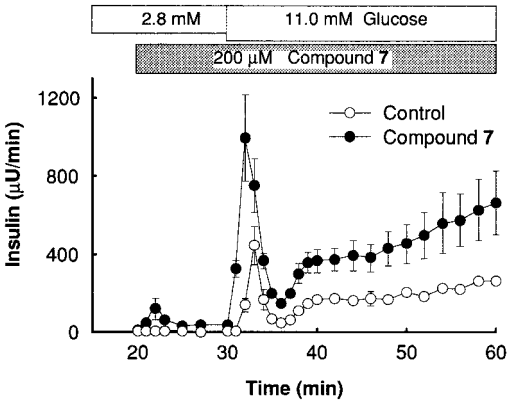



**Figure 3.** Effects of 200 $\mu$M compound **7** on insulin secretory response to 2.8 mM glucose or 11.1 mM glucose of perfused pancreases isolated from diabetic nSTZ Sprague−Dawley rats (14 weeks old). Values are the mean ± SE. ($N = 4$).

In summary, we determined the structural requirements for the antidiabetic activity of 4-cycloalkyl-4-oxobutyric acids and their analogues, and obtained 4-(*trans*-4-methylcyclohexyl)-4-oxobutylic acid **7** as the optimum compound. This compound caused a marked improvement of glucose intolerance and the $\beta$-cell response to glucose in diabetic nSTZ rats. Selective improvement of glucose-induced insulin secretion was recognized in studies using nSTZ rats and perfused pancreases isolated from nSTZ rats. Since these findings suggested that compound **7** could be useful for improving glucose homeostasis in type 2 diabetes, it is currently undergoing evaluation in clinical trials. The

biological response to compound **7** and the strict structural specificity of the active compound may indicate the existence of a specific binding site and a related system that modulates the response of pancreatic $\beta$-cells to glucose. Further studies on compound **7** may help to elucidate the mechanism by which $\beta$-cells respond to glucose and the relationship between this mechanism and type 2 diabetes.

## Experimental Section

**Chemistry.** Melting points were obtained with a Yanagimoto micro melting point apparatus or a Mettler-Toledo FP62 melting point instrument and are uncorrected. Elemental analysis was performed with a Perkin-Elmer 2400 Series II CHNS/O analyzer, and all values were within ±0.4% of the calculated values. FAB mass spectra were recorded on a Finnigan TSQ 700 spectrometer. High-resolution mass spectra were obtained with a JEOL SX 102A spectrometer. $^1$H NMR spectra were recorded on a JEOL JNM-A300W, Bruker AMX 300, or Bruker ARX 400 spectrometer in a solution of either $CDCl_3$, $CD_3OD$, or DMSO-$d_6$, using tetramethylsilane as the internal standard. Chemical shifts are expressed as $\delta$ (ppm) values for protons relative to the internal standard; all compounds gave spectra consistent with their assigned structures.

**Di-*tert*-butyl 2-(ethoxycabonylmethyl)malonate (20).** A solution of di-*tert*-butyl malonate (96.6 g, 447 mmol) in anhydrous tetrahydrofuran (150 mL) was added to a suspension of sodium hydride (18 g, 450 mmol, 60% dispersion) in anhydrous tetrahydrofuran (500 mL) at room temperature. When evolution of hydrogen ceased, a solution of ethyl bromoacetate (57.2 g, 343 mmol) in anhydrous tetrahydrofuran (250 mL) was added, and the mixture was stirred for 4 h. Then the mixture was poured into saturated aqueous ammonium chloride (500 mL) and extracted with ethyl acetate (200 mL × 2). The combined extract was washed with brine (500 mL), dried over sodium sulfate, and concentrated. The residue was distilled to give **20** (69.8 g, 67%): $bp_{0.8}$ 115 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.25 (3H, t, $J$ = 6.9 Hz), 1.46 (18H, s), 1.81 (2H, d, $J$ = 7.3 Hz), 3.64 (1H, t, $J$ = 7.3 Hz), 4.15 (2H, q, $J$ = 7.1 Hz); MS (FAB) $m/z$ 303 $(M + H)^+$. HRMS calcd for $C_{15}H_{27}O_6$, 303.1808; found, 303.1809.

**4-Oxobutyric Acids (1−7 and 9−11).** A typical run (**7** in Table 1) was as follows. A solution of **20** (68.3 g, 226 mmol) in anhydrous tetrahydrofuran (642 mL) was added to a suspension of sodium hydride (9.49 g, 237 mmol, 60% dispersion) in anhydrous tetrahydrofuran (100 mL) at room temperature. When evolution of hydrogen ceased, a solution of 4-methylcyclohexanecarbonyl chloride (36.3 g, 226 mmol) in anhydrous tetrahydrofuran (250 mL) was added, and the mixture was stirred for 2 h. Then the mixture was poured into saturated aqueous ammonium chloride (500 mL) and extracted with ethyl acetate (200 mL × 2). The combined extract was washed with brine (500 mL), dried over sodium sulfate, and concentrated to a crude residue. *p*-Toluenesulfonic acid monohydrate (4.08 g, 21.4 mmol) was added to a solution of the product (91.5 g, 215 mmol) in toluene (914 mL), and the mixture was refluxed for 2 h. After cooling to room temperature, the mixture was washed with saturated aqueous sodium hydrogencarbonate (300 mL) and water (300 mL). The organic layer was dried over sodium sulfate and concentrated to give ethyl 4-(4-methylcyclohexyl)-4-oxobutyrate as an oil (46.7 g, 92%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.88−0.98 (2H, m), 0.89 (3H, d, $J$ = 6.5 Hz), 1.22 (3H, t, $J$ = 7.3 Hz), 1.25−1.43 (3H, m), 1.74−1.80 (2H, m), 1.86−1.92 (2H, m), 2.32 (1H, tt, $J$ = 3.3 and 12.7 Hz), 2.56 (2H, t, $J$ = 6.5 Hz), 2.75 (2H, t, $J$ = 6.6 Hz), 4.12 (2H, q, $J$ = 7.4 Hz). To a solution of the oil (46.7 g, 206 mmol) in ethanol (500 mL) was added 1 N aqueous sodium hydroxide (226.7 mL), and the mixture was stirred at room temperature for 12 h. Then the mixture was acidified with 1 N hydrochloric acid, and the solvent was removed under a vacuum. The residue was dissolved in ethyl acetate (500 mL) and washed with brine (200 mL), after which the solution was dried over sodium sulfate and concentrated. The crude product was crystallized from ethyl acetate/hexane, followed by recrystallization from aqueous methanol to give 4-(*trans*-4-methylcyclohexyl)-4-oxobutyric acid **7** as a white solid (19.7 g, 48%): mp 100.4−101.2 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.88−1.02 (2H, m), 0.89 (3H, d, $J$ = 6.5 Hz), 1.26−1.43 (1H, m), 1.36 (2H, dq, $J$ = 3.3 and 12.7 Hz), 1.78 (2H, dd, $J$ = 3.3 and 13.5 Hz), 1.89 (2H, m), 2.31 (1H, tt, $J$ = 3.3 and 12.7 Hz), 2.62 (2H, t, $J$ = 6.3 Hz), 2.76 (2H, t, $J$ = 6.3 Hz). Anal. ($C_{11}H_{18}O_3$) C, H.

**4-Cyclohexyl-4-oxobutyric Acid (1):** mp 73.8−74.9 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.17−1.43 (5H, m), 1.64−1.69 (1H, m), 1.77−1.88 (4H, m), 2.31−2.40 (1H, m), 2.62 (2H, t, $J$ = 6.2 Hz), 2.75 (2H, t, $J$ = 6.2 Hz); MS (FAB) $m/z$ 185 $(M + H)^+$. HRMS calcd for $C_{10}H_{17}O_3$, 185.1178; found, 185.1183. Anal. ($C_{10}H_{16}O_3$) C, H.

**4-Cyclopentyl-4-oxobutyric Acid (2):** mp 79.8 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.53−1.88 (8H, m), 2.63 (2H, t, $J$ = 7.0 Hz), 2.77 (2H, t, $J$ = 7.0 Hz), 2.90 (1H, quintet, $J$ = 8.0 Hz); MS (FAB) $m/z$ 171 $(M + H)^+$. HRMS calcd for $C_9H_{15}O_3$, 171.1021; found, 171.1011. Anal. ($C_9H_{14}O_3$) C, H.

**4-Cycloheptyl-4-oxobutyric Acid (3):** mp 48.7 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 1.43−1.82 (12H, m), 2.38 (2H, t, $J$ = 6.4 Hz), 2.53−2.62 (1H, m), 2.68 (2H, t, $J$ = 6.4 Hz), 2.68 (2H, t, $J$ = 6.4 Hz), 12.03 (1H, br s); MS (FAB) $m/z$ 199 $(M + H)^+$. HRMS calcd for $C_{11}H_{19}O_3$, 199.1334; found, 199.1327. Anal. ($C_{11}H_{18}O_3$) C, H.

**4-(1-Methylcyclohexyl)-4-oxobutyric Acid (4):** mp 66.8−67.2 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.10 (3H, s), 1.26−1.41 (8H, m), 1.93−2.00 (2H, m), 2.62 (2H, t, $J$ = 6.0 Hz), 2.81 (2H, t, $J$ = 6.0 Hz); MS (FAB) $m/z$ 199 $(M + H)^+$. HRMS calcd for $C_{11}H_{19}O_3$, 199.1334; found, 199.1321. Anal. ($C_{11}H_{18}O_3$) C, H.

**4-(2-Methylcyclohexyl)-4-oxobutyric Acid (5):** mp 70.4−70.6 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.81 (3H, d, $J$ = 6.5 Hz), 0.94−1.03 (1H, m), 1.18−1.35 (3H, m), 1.63−1.82 (5H, m), 2.08−2.17 (1H, m), 2.59−2.63 (2H, m), 2.67−2.71 (1H, m), 2.78−2.89 (1H, m); MS (FAB) $m/z$ 199 $(M + H)^+$. HRMS calcd for $C_{11}H_{19}O_3$, 199.1334; found, 199.1340. Anal. ($C_{11}H_{18}O_3$) C, H.

**4-(3-Methylcyclohexyl)-4-oxobutyric Acid (6):** mp 56.0−57.0 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.80−1.06 (2H, m), 0.92 (3H, d, $J$ = 6.6 Hz), 1.21−1.49 (3H, m), 1.66−1.72 (1H, m), 1.79−1.89 (3H, m), 2.34−2.47 (1H, m), 2.62 (2H, t, $J$ = 6.6 Hz), 2.76 (2H, t, $J$ = 6.6 Hz); MS (FAB) $m/z$ 199 $(M + H)^+$. HRMS calcd for $C_{11}H_{19}O_3$, 199.1334; found, 199.1340. Anal. ($C_{11}H_{18}O_3$) C, H.

**4-(4,4-Dimethylcyclohexyl)-4-oxobutyric Acid (9).** 4,4-Dimethyl-cyclohexanecarbonyl chloride, which was a starting material for **9**, was prepared by the reported method[13]: mp 50.0 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.89 (3H, s), 0.92 (3H, s), 1.20 (2H, dt, $J$ = 3.8 and 13.1 Hz), 1.43−1.63 (4H, m), 1.68−1.74 (2H, m), 2.29 (1H, tt, $J$ = 3.4 and 12.1 Hz), 2.62 (2H, t, $J$ = 6.3 Hz), 2.77 (2H, t, $J$ = 6.3 Hz); MS (FAB) $m/z$ 213 $(M + H)^+$. HRMS calcd for $C_{12}H_{21}O_3$, 213.1491; found, 213.1483. Anal. ($C_{12}H_{20}O_3$) C, H.

**4-(*trans*-4-Ethylcyclohexyl)-4-oxobutyric Acid (10):** mp 103.0−104.0 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.85−0.97 (2H, m), 0.88 (3H, t, $J$ = 7.4 Hz), 1.03−1.41 (5H, m), 1.82−1.93 (4H, m), 2.32 (1H, tt, $J$ = 3.3 and 12.2 Hz), 2.62 (2H, t, $J$ = 6.6 Hz), 2.76 (2H, t, $J$ = 6.6 Hz). Anal. ($C_{12}H_{20}O_3$) C, H.

**4-(*trans*-4-Propylcyclohexyl)-4-oxobutyric Acid (11):** mp 109.7−113.5 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.85−0.99 (2H, m), 0.88 (3H, t, $J$ = 7.1 Hz), 1.15−1.40 (7H, m), 1.80−1.94 (4H, m), 2.32 (1H, tt, $J$ = 3.3 and 12.7 Hz), 2.62 (2H, t, $J$ = 6.5 Hz), 2.76 (2H, t, $J$ = 6.6 Hz); MS (FAB) $m/z$ 227 $(M + H)^+$. HRMS calcd for $C_{13}H_{23}O_3$, 227.1647; found, 227.1646. Anal. ($C_{13}H_{22}O_3$) C, H.

**Benzyl 4-(*cis*-4-Methylcyclohexyl)-4-oxobutyrate (24).** Benzyl bromide (10.8 g, 63.2 mmol) and sodium hydrogencarbonate (5.31 g) were added to a solution of **22**, which was obtained from the mother liquor after separating trans isomer **7** by crystallization in *N*,*N*-dimethylformamide (100 mL). The mixture was stirred for 12 h and then evaporated under a vacuum. The residue was dissolved in ethyl acetate (100 mL) and washed with saturated aqueous sodium hydrogencarbon-

ate (30 mL) and brine (30 mL). The solution was dried over sodium sulfate and concentrated, after which the crude oil was chromatographed on a silica gel column eluted with hexane/ethyl acetate (10:1) to give **23** (7.73 g, 85%). A mixture of the cis and trans isomers in a 1:1 ratio (**23**, 7.73 g, 26.8 mmol) was purified by HPLC using a YMC SIL-5-B-06 column (30 mm × 250 mm i.d.) developed with a mixture of *n*-hexane and ethyl acetate in a 93:7 ratio at a flow rate of 15 mL/min, yielding **24** as a colorless oil (3.48 g, 21.1 mmol): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.89 (3H, d, $J$ = 6.6 Hz), 1.18−1.29 (2H, m), 1.48−1.64 (5H, m), 1.84−1.95 (2H, m), 2.47 (1H, tt, $J$ = 4.5 and 6.6 Hz), 2.64 (2H, t, $J$ = 6.9 Hz), 2.79 (2H, t, $J$ = 6.9 Hz), 5.11 (2H, s), 7.28−7.39 (5H, m); MS (FAB) *m*/*z* 289 $(M + H)^+$. HRMS calcd for $C_{18}H_{25}O_3$: 289.1804; found, 289.1812.

**4-(*cis*-4-Methylcyclohexyl)-4-oxobutyric Acid (8).** A solution of **24** (3.48 g, 12.1 mmol) in ethyl acetate (40 mL) was stirred in the presence of 10% palladium on charcoal (350 mg) under an atmosphere of hydrogen at room temperature until hydrogen uptake ceased. The solution was filtered through Celite, and the filtrate was evaporated to give **8** as a white solid (2.20 g, 91.7%): mp 49.3 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.90 (3H, d, $J$ = 6.7 Hz), 1.20−1.30 (2H, m), 1.49−1.63 (5H, m), 1.87−1.98 (2H, m), 2.48 (1H, tt, $J$ = 4.6 and 6.4 Hz), 2.63 (2H, t, $J$ = 6.6 Hz), 2.76 (2H, t, $J$ = 6.6 Hz); MS (FAB) *m*/*z* 199 $(M + H)^+$. HRMS calcd for $C_{11}H_{19}O_3$, 199.1334; found, 199.1349. Anal. ($C_{11}H_{19}O_3$) C, H.

**4-(*trans*-4-Methylcyclohexyl)butyric Acid (12).** A solution of **7** (1.98 g, 10 mmol), hydrazine (1.62 mL, 33 mmol), and potassium hydroxide (1.85 g, 33 mmol) in ethylene glycol (10 mL) was refluxed for 4 h. Then water (20 mL) and 6 N hydrochloric acid (10 mL) were added to the mixture, and it was extracted with hexane (2 × 30 mL). The combined extracts were washed with water (2 × 10 mL) and brine (10 mL), dried over sodium sulfate, and concentrated. Chromatography of the residue on a silica gel column eluted with ethyl acetate/hexane (3:7) gave a brown solid. The crude solid was crystallized from ethanol/water (1:4) to give **12** as a light yellow solid (0.59 g, 32%): mp 55.0 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.86 (3H, d, $J$ = 6.0 Hz), 0.80−1.00 (4H, m), 1.05−1.40 (5H, m), 1.55−1.80 (5H, m), 2.33 (1H, t, 7.8 Hz); MS (FAB) *m*/*z* 185 $(M + H)^+$. HRMS calcd for $C_{11}H_{21}O_2$, 185.1541; found, 185.1536. Anal. ($C_{11}H_{20}O_2$) C, H.

**Ethyl 3-(*trans*-4-Methylcyclohexylthio)propionate (25).** Ethyl 3-mercaptopropionate (4.00 g, 29.8 mmol) and cesium carbonate (12.14 g, 37.3 mmol) were added to a solution of the tosylate (5.00 g, 18.6 mmol) in acetonitrile (150 mL) and stirred at 50 °C for 4 h. After the mixture was cooled to room temperature, the precipitate was removed by filtration and the organic solvent was evaporated. The residual solvent was diluted with water (50 mL) and extracted with ethyl acetate (50 mL), after which the extract was washed with brine (20 mL), dried over sodium sulfate, and concentrated. The crude oil was chromatographed on a silica gel column eluted with hexane/ethyl acetate (15:1) to give **25** as a colorless oil (1.12 g, 26%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.87−1.03 (2H, m), 0.88 (3H, d, $J$ = 6.5 Hz), 1.24−1.40 (3H, m), 1.26 (3H, t, $J$ = 7.1 Hz), 1.53−1.57 (2H, m), 1.72−1.76 (2H, m), 2.51−2.93 (3H, m), 2.58 (2H, t, $J$ = 7.6 Hz), 4.16 (2H, q, $J$ = 7.1 Hz); MS (FAB) *m*/*z* 231 $(M + H)^+$. HRMS calcd for $C_{12}H_{23}O_2S$, 231.1419; found, 231.1412.

**3-(*trans*-4-Methylcyclohexylsulfinyl)propionic Acid (13).** *m*-Chloroperoxybenzolic acid (1.2 g, 6.95 mmol) was added in aliquots to a solution of **25** (1.12 g, 4.86 mmol) in chloroform (20 mL) at 0 °C. After 30 min, calcium hydroxide (360 mg, 4.86 mmol) was added to the mixture, which was stirred at room temperature for 10 min. After removal of the precipitate by filtration, the filtrate was concentrated. The residue was dissolved in ethyl acetate (20 mL) and washed with saturated aqueous sodium hydrogencarbonate (10 mL) and brine (10 mL). Then the solution was dried over sodium sulfate and concentrated. The crude residue was chromatographed on silica gel eluted with hexane/ethyl acetate (15:1) to give a white solid (686 mg, 57%). A solution of lithium hydroxide monohydrate (91 mg, 2.18 mmol) in water (0.5 mL) was added to a solution of the white solid (269 mg, 1.09 mmol) in ethanol (1.5 mL). The mixture was stirred at room temperature for 30 min and then acidified with 1 N hydrochloric acid. After evaporation of the solvent, the residue was dissolved in ethyl acetate (5 mL) and washed with saturated aqueous sodium hydrogencarbonate (2 mL) and brine (2 mL). The solution was dried over sodium sulfate and concentrated. Then the crude residue was chromatographed on a silica gel column eluted with hexane/ethyl acetate (15:1) to give **13** as a white solid (135 mg, 54%): mp 88.7−89.5 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.92 (3H, d, $J$ = 6.6 Hz), 0.93−1.08 (2H, m), 1.35−1.53 (3H, m), 1.86−1.94 (3H, m), 2.18−2.22 (2H, m), 2.62 (1H, br t, $J$ = 12.5 Hz), 2.86−2.91 (2H, m), 2.98−3.06 (2H, m); MS (FAB) *m*/*z* 219 $(M + H)^+$. HRMS calcd for $C_{10}H_{19}O_3S$, 219.1055; found, 219.1068. Anal. ($C_{10}H_{18}O_3S$) C, H.

**3-(*trans*-4-Methylcyclohexylsulfonyl)propionic Acid (14).** *m*-Chloroperoxybenzolic acid (1.32 g, 7.36 mmol) was added in aliquots to a solution of **25** (0.615 g, 267 mmol) in chloroform (20 mL) at 0 °C. After 30 min, calcium hydroxide (665 mg, mol) was added to the mixture, which was stirred at room temperature for 10 min. After removal of the precipitate by filtration, the filtrate was concentrated. Then the residue was dissolved in ethyl acetate (15 mL) and washed with saturated aqueous sodium hydrogencarbonate (5 mL) and brine (5 mL). The resulting solution was dried over sodium sulfate and concentrated. The crude residue was chromatographed on silica gel eluted with hexane/ethyl acetate (15:1) to give a white solid (659 mg, 94%). Next, 12 N hydrochloric acid (6 mL) was added to a solution of the white solid (659 mg, 2.51 mmol) in 1,4-dioxane (1 mL), and the mixture was refluxed for 12 h. After the mixture was cooled to room temperature, the solvent was evaporated under a vacuum. The residue was dissolved in ethyl acetate (5 mL) and washed with 5% aqueous potassium hydrogensulfate (3 mL) and brine (3 mL), after which the solution was dried over sodium sulfate and concentrated. The crude residue was chromatographed on silica gel eluted with hexane/ethyl acetate (15:1) to give **14** as a white solid (560 mg, 95%): mp 145.1 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.93 (3H, d, $J$ = 6.6 Hz), 0.99 (2H, dq, $J$ = 3.3 and 12.6 Hz), 1.33−1.50 (1H, m), 1.61 (2H, dq, $J$ = 3.3 and 12.8 Hz), 1.91 (2H, d, $J$ = 12.0 Hz), 2.20 (2H, d, $J$ = 12.0 Hz), 2.83 (1H, tt, $J$ = 3.5 and 12.2 Hz), 2.94 (2H, t, $J$ = 7.5 Hz), 3.25 (2H, t, $J$ = 7.5 Hz); MS (FAB) *m*/*z* 235 $(M + H)^+$. HRMS calcd for $C_{10}H_{19}O_4S$, 235.1004; found, 235.1014. Anal. ($C_{10}H_{18}O_4S$) C, H.

**[(*trans*-4-Methylcyclohexanecarbonyl)oxy]acetic Acid (15).** *tert*-Butyl bromoacetate (3.02 g, 15.5 mmol) and potassium carbonate (3.98 g, 28.8 mmol) were added to a solution of *trans*-4-methylcyclohexanecarboxylic acid (2.0 g, 14.1 mmol) in acetone (100 mL). The mixture was refluxed for 3 h and then cooled to room temperature. Then the mixture was diluted with ether (200 mL), washed with water (100 mL) and brine (100 mL), and concentrated to a colorless oil (3.6 g, 99%). Trifluoroacetic acid (10 mL) was added to a solution of the oil (2.0 g, 7.80 mmol), after which the mixture was stirred for 1 h and concentrated. The residue was chromatographed on a silica gel column eluted with hexane/ethyl acetate (15:1) to give **15** as a white solid (1.14 g, 73%): mp 99.4 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.89 (3H, d, $J$ = 6.5 Hz), 0.95 (2H, dq, $J$ = 3.4 and 13.3 Hz), 1.30−1.50 (1H, m), 1.47 (2H, dq, $J$ = 3.4 and 12.8 Hz), 1.77 (2H, d, $J$ = 10.4 Hz), 2.01 (2H, d, $J$ = 10.4 Hz), 2.33 (1H, tt, $J$ = 3.6 and 12.2 Hz), 4.65 (2H, s); MS (FAB) *m*/*z* 201 $(M + H)^+$. HRMS calcd for $C_{10}H_{17}O_4$, 201.1127; found, 201.1130. Anal. ($C_{10}H_{16}O_4$) C, H.

**[(*trans*-4-Methylcyclohexanecarbonyl)amino]acetic Acid (16).** After a mixture of *trans*-4-methylcyclohexanecarboxylic acid (0.853 g, 6.0 mmol), 1-hydroxybenzotriazole (0.891 g, 6.60 mmol), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (1.207 g, 6.30 mmol) in *N*,*N*-dimethylformamide (10 mL) was stirred for 20 min, glycine benzyl ester *p*-toluenesulfonate (2.23 g, 6.60 mmol) and *N*-methylmorpholine (1.33 g, 13.2 mmol) were added, and the mixture was stirred for 2 h. Then the mixture was diluted with 1 N hydrochloric acid (30 mL) and extracted with ethyl acetate (20

mL × 3). The extract was washed with water (20 mL), saturated aqueous sodium hydrogencarbonate (20 mL), and brine (20 mL), and the resluting solution was dried over sodium sulfate and concentrated to a white solid (1.66 g, 96%). A solution of the white solid (1.66 g, 5.74 mmol) in ethanol (50 mL) was stirred in the presence of 5% palladium on charcoal (1.52 g) under an atmosphere of hydrogen at room temperature until hydrogen uptake ceased. The solution was filtered through Celite, and the filtrate was evaporated to give **16** as a white solid (1.13 g, 99%): mp 149.2 °C; $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 0.89 (3H, d, $J$ = 6.6 Hz), 0.97 (2H, dq, $J$ = 3.3 and 12.5 Hz), 1.30−1.40 (1H, m), 1.46 (2H, dq, $J$ = 3.3 and 12.5 Hz), 1.76 (2H, dd, $J$ = 3.1 and 13.5 Hz), 1.84 (2H, dd, $J$ = 3.1 and 13.5 Hz), 2.16 (1H, tt, $J$ = 3.4 and 12.2 Hz), 3.87 (2H, d, $J$ = 4.0 Hz); MS (FAB) $m/z$ 200 $(M + H)^+$. HRMS calcd for $C_{10}H_{18}NO_3$, 200.1287; found, 200.1291. Anal. ($C_{10}H_{17}NO_3$) C, H, N.

**2-Bromo-1-(*trans*-4-methylcyclohexyl)-1-ethanone (27).** Methyllithium (1.4 M) in ether (48 mL, 207 mmol) was added dropwise to a solution of *trans*-4-methylcyclohexanecarboxylic acid (12.9 g, 90.7 mmol) in anhydrous ether (700 mL) at 0 °C. After being warmed to room temperature, the mixture was stirred for 4 h and then poured into water (500 mL). The organic layer was washed with saturated aqueous sodium hydrogencarbonate (300 mL) and brine (300 mL), after which the solution was dried over sodium sulfate and concentrated to a colorless oil (9.47 g, 74%). The crude product was used for the next step without purification. Bromine (11.3 g, 70.9 mmol) was added to a solution of the colorless oil obtained as above (9.47 g, 67.5 mmol) in methanol (60 mL) at 0 °C, and the mixture was stirred for 30 min. Then the mixture was poured into water (150 mL) and extracted with ether (50 mL × 2). The combined organic layers were dried over sodium sulfate and concentrated to give **27** as a colorless oil (14.1 g, 95%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.90 (3H, d, $J$ = 6.0 Hz), 0.97 (2H, dq, $J$ = 3.0 and 12.0 Hz), 1.23−1.48 (1H, m), 1.41 (2H, dq, $J$ = 3.0 and 12.0 Hz), 1.79 (2H, dd, $J$ = 3.0 and 12.0 Hz), 1.90 (2H, dd, $J$ = 3.0 and 12.0 Hz), 2.64 (1H, tt, $J$ = 3.0 and 12.0 Hz), 3.97 (2H, s); MS (FAB) $m/z$ 219 and 221 $(M + H)^+$. HRMS calcd for $C_9H_{16}OBr$, 219.0384 and 221.0364; found, 219.0393 and 221.0391.

**1-(*trans*-4-Methylcyclohexyl)-1,4-pentanedione (17).** Benzyl acetoacetate (3.16 g, 16.4 mmol) was added to a suspension of sodium hydride (0.69 g, 17.3 mmol, 60% dispersion) in anhyrous tetrahydrofuran (60 mL) at 0 °C. When evolution of hydrogen ceased, a solution of **27** (4.0 g, 18.3 mmol) in anhydrous tetrahydrofuran (20 mL) was added, and the mixture was stirred at room temperature for 1 h. Then the solution was poured into saturated aqueous ammonium chloride (50 mL) and extracted with ethyl acetate (30 mL × 2). The combined extracts were washed with brine (30 mL), dried over sodium sulfate, and concentrated to give benzyl 2-acetyl-4-(*trans*-4-methylcyclohexyl)-4-oxobutyrate (**28**) as a colorless oil (3.96 g, 69%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.86−0.98 (2H, m), 0.88 (3H, d, $J$ = 6.0 Hz), 1.31 (2H, dq, $J$ = 3.0 and 15.0 Hz), 1.72−1.80 (2H, m), 1.83−1.92 (2H, m), 2.23−2.32 (1H, m), 2.30 (3H, s), 2.96 (1H, dd, $J$ = 6.0 and 18.0 Hz), 3.17 (1H, dd, $J$ = 9.0 and 18.0 Hz), 4.07 (1H, dd, $J$ = 6.0 and 9.0 Hz), 5.16 (2H, s), 7.33−7.38 (5H, m). A solution of **28** (3.96 g, 11.98 mmol) in ethanol (59 mL) was stirred in the presence of 10% palladium on charcoal (0.719 g) under an atmosphere of hydrogen at room temperature for 1 h. Then the solution was filtered through Celite and the filtrate was evaporated under a vacuum. The residue was chromatographed on a silica gel column eluted with hexane/ethyl acetate (15:1) to give **17** as a colorless oil (2.0 g, 85%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.89 (3H, d, $J$ = 6.0 Hz), 0.88−1.01 (2H, m), 1.28−1.41 (1H, m), 1.35 (2H, dq, $J$ = 3.0 and 12.0 Hz), 1.75−1.80 (2H, m), 1.87−1.92 (2H, m), 2.18 (3H, s), 2.32 (1H, tt, $J$ = 4.5 and 12.0 Hz), 2.67−2.73 (4H, m); MS (FAB) $m/z$ 197 $(M + H)^+$. HRMS calcd for $C_{12}H_{21}O_2$, 197.1541; found, 197.1524. Anal. ($C_{12}H_{20}O_2$) C, H.

**5-(*trans*-4-Methylcyclohexyl)-5-oxovaleric Acid (19).** *n*-Butyllithium (1.6 M) in hexane (33 mL, 52.9 mmol) was added dropwise to a solution of ethyl hydrogen malonate (3.5 g, 26.5 mmol) in anhydrous tertahydrofuran (80 mL) at −78 °C, and was slowly warmed to −30 °C. After the mixture was cooled to −78 °C again, 4-methylcyclohexanecarbonyl chloride (2.0 g, 12.5 mmol) was added dropwise to the mixture. Then the solution was warmed to room temperature and stirred for 1 h. Next, the solution was poured into 1 N hydrochloric acid (50 mL) extracted with ether (30 mL). The combined extracts were washed with saturated aqueous sodium hydrogencarbonate (30 mL) and brine (30 mL), dried over sodium sulfate, and concentrated to give ethyl (4-methylcyclohexanecarbonyl)-acetate (**29**) as a colorless oil (2.38 g, 89.6%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.90 (3H, d, $J$ = 6.6 Hz), 1.20−1.94 (9H, m), 1.28 (3H, t, $J$ = 7.1 Hz), 2.54−2.63 (1H, m), 2.48 (2H, s), 4.19 (2H, q, $J$ = 7.1 Hz). A solution of **29** (2.11 g, 9.94 mmol) in *tert*-butyl alcohol (30 mL) was added to a solution of potassium *tert*-butoxide (1.23 g, 11.0 mmol) in *tert*-butyl alcohol (10 mL), and the mixture was stirred for 30 min. Ethyl 3-bromopropionate (1.9 g, 10.5 mmol) was added dropwise to the mixture, which was stirred for 2 h. Then the reaction mixture was poured into 1 N hydrochloric acid (50 mL), extracted with ethyl acetate (30 mL), washed with brine (10 mL), dried over sodium sulfate, and concentrated. The residue was chromatographed on a silica gel column eluted with hexane/ethyl acetate (15:1) to give diethyl (4-methylcyclohexanecarbonyl)-succinate (**30**) as a colorless oil (3.0 g, 97%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.91 (3H, d, $J$ = 6.8 Hz), 1.23−1.95 (9H, m), 1.25 (3H, t, $J$ = 7.2 Hz), 2.13 (2H, t, $J$ = 7.0 Hz), 2.33 (2H, t, $J$ = 7.3 Hz), 2.63−2.71 (1H, m), 3.79 (2H, t, $J$ = 7.1 Hz), 4.10−4.21 (4H, m). A 1 N sodium hydroxide solution (17 mL) was added to a solution of **30** (1.35 g, 4.32 mmol) in methanol (50 mL). The mixture was stirred at room temperature for 12 h, and then the solvent was removed under a vacuum. Next, the residue was diluted with ethyl acetate (30 mL), washed with 1 N hydrochloric acid (10 mL) and brine (10 mL), dried over sodium sulfate, and concentrated to a colorless oil (1.07 g, 4.19 mmol). This oil was heated at 100 °C for 40 min and cooled to room temperature to give a white solid (0.84 g, 3.96 mmol). The crude product was crystallized from ethyl acetate/hexane (2:8) to give **19** as a white solid (0.582 g, 2.74 mmol): mp 78.2−80.0 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.87−1.00 (2H, m), 0.89 (3H, d, $J$ = 6.0 Hz), 1.26−1.40 (3H, m), 1.74−1.94 (4H, m), 1.89 (2H, quintet, $J$ = 7.1 Hz), 2.26 (1H, tt, $J$ = 3.3 and 12.2 Hz), 2.38 (2H, t, $J$ = 7.1 Hz), 2.53 (2H, t, $J$ = 7.1 Hz); MS (FAB) $m/z$ 213 $(M + H)^+$. HRMS calcd for $C_{12}H_{21}O_3$, 213.1491; found, 213.1483. Anal. ($C_{12}H_{20}O_3$) C, H.

**4-(*trans*-4-Methylcyclohexyl)-4-oxobutyramide (18):** mp 111.3 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.88−1.00 (2H, m), 0.89 (3H, d, $J$ = 6.5 Hz), 1.27−1.40 (1H, m), 1.34 (2H, dq, $J$ = 3.3 and 12.3 Hz), 1.77 (2H, dd, $J$ = 3.2 and 13.4 Hz), 1.89 (2H, d, $J$ = 12.1 Hz), 2.31 (1H, tt, $J$ = 3.4 and 12.1 Hz), 2.47 (2H, t, $J$ = 6.5 Hz), 2.82 (2H, t, $J$ = 6.5 Hz), 5.37 (1H, br s), 5.68 (1H, br s); MS (FAB) $m/z$ 198 $(M + H)^+$. HRMS calcd for $C_{11}H_{20}NO_2$, 198.1494; found, 198.1493. Anal. ($C_{11}H_{19}NO_2$) C, H, N.

**Pharmacology.** The effect of the compounds on fasting euglycemia and glucose tolerance was evaluated in 7-week-old male Wistar rats (Charles River Japan, Tokyo, Japan) and 10-week-old diabetic nSTZ rats. nSTZ rats were created by giving a single subcutaneous injection of 120 mg/kg of streptozotocin to neonetal (1.5-day-old) male Sprague−Dawley rats (Charles River Japan, Tokyo, Japan).

**Effect on Fasting Euglycemia.** Test animals (in groups of four or five) were fasted for 16 h and then dosed orally with either the vehicle (control) or active compounds (10, 30, or 100 mg/kg). Blood samples were taken from the tail vein over a 4 h period for measurement of the blood glucose and insulin levels.

**Effect on Glucose Tolerance.** Test animals (in groups of four to six) were fasted for 16 h and then dosed intraperitoneally (1 g/kg) or orally (2 g/kg) with glucose, as well as orally with either the vehicle (control) or active compounds (3 to 30 mg/kg). Blood samples were taken from the tail vein over a 4 h period for measurement of blood glucose and insulin levels. The blood glucose level was measured by the hexokinase

method[14] using a commercial kit (Boehringer Mannheim, Tokyo, Japan), and the insulin level was measured by the two-antibody method[15] using a radioimmunoassay kit (Shionogi, Osaka, Japan). Statistical evaluation of the results was done with Dunnett's two-tailed test (*$p < 0.05$, **$p < 0.01$).

**Effect on Insulin Secretion by Perfused Pancreases.** Isolation and perfusion of rat pancreases was performed according to the method of Grodski with minor modifications.[16] After overnight fasting, the pancreas, spleen, and duodenum were harvested from 7-week-old normal Wistar rats or 14-week-old diabetic nSTZ Sprague−Dawley rats under sodium pentobarbital anesthesia (50 mg/kg, ip). Each isolated pancreas was perfused through the celiac artery at a flow rate of 3.5 mL/min with basal Krebs−Ringer bicarbonate buffer (KRB buffer: 119 mM NaCl, 4.74 mM KCl, 2.54 mM $CaCl_2$, 1.19 mM $MgCl_2$, 1.19 mM $KH_2PO_4$, 25 mM $NaHCO_3$, pH 7.4; equilibrate with 95% $O_2$ and $CO_2$) containing 2.8 mM glucose, 0.2% bovine serum albumin (fraction V, Sigma, St. Louis), and 4.0% dextran T-70 (Pharmacia Biotech, Uppsala, Sweden). Each pancreas was placed in an acrylic chamber filled with KRB buffer and maintained at 37 °C. After equilibration for 20 min, the perfusate was changed to KRB buffer containing a test compound for 10 min and then to KRB buffer containing the test compound and high concentration of glucose (7.0 or 11.1 mM) for 30 min. Effluent from the portal vein cannula was collected at 1 min intervals into tubes containing aprotine (1000 U/tube). The insulin concentration was measured with a radioimmunoassay kit (Pharmacia Upjohn, Uppsala, Sweden) using rat insulin as the standard.[15] Results are shown as the mean ± SE. ($N = 3-4$).

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