Discovery of a Long-Chain Carbamoyl Aminocarnitine Derivative, a Reversible Carnitine Palmitoyltransferase Inhibitor with Antiketotic and Antidiabetic Activity

Fabio Giannessi,*,† Pompeo Pessotto,‡ Emanuela Tassoni,† Piero Chiodi,‡ Roberto Conti,‡ Francesco De Angelis,§ Natalina Dell'Uomo,† Roberto Catini,† Roberto Deias,|| Maria Ornella Tinti,† Paolo Carminati,‡ and Arduino Arduini‡,⊥

Departments of Chemical Research, Endocrinology & Metabolism, and Analytical Research, Sigma Tau Pharmaceutical Industries S.p.A., Via Pontina Km 30.400, 00040 Pomezia, Italy, and Department of Chemistry, Chemical Engineering and Materials, University of L'Aquila, L'Aquila, Italy

Received June 27, 2002

The synthesis and pharmacological activity of reversible CPT I inhibitors as potential antiketotic and antidiabetic drugs are reported. Such inhibitors constitute a series of enantiomerically pure aminocarnitine derivatives having the general formula $(CH_3)_3N^+CH_2CH(ZR)CH_2COO^-$ (with Z = ureido, carbamate, sulfonamide, and sulfamide moieties; R = C_7−C_{14} linear alkyl chains). A primary pharmacological screening based on the evaluation of CPT I activity in intact rat liver (L-CPT I) mitochondria revealed the best activity for the (*R*) forms of ureidic derivative **17** (ZR = NHCONHR, R = C_{14}), sulfonamidic derivative **7** (ZR = $NHSO_2R$, R = C_{12}), and sulfamidic derivative **9** (ZR = $NHSO_2NHR$, R = C_{11}). The IC_{50} values are 1.1, 0.7, and 0.8 μM, respectively. For the carbamic derivative **11** (ZR = NHCOOR, R = C_8), an IC_{50} of 9.5 μM was observed. In addition, an extraordinarily high selectivity toward the liver isoform with respect to the heart isoform (muscle-CPT I ≡ M-CPT I) was found for the ureidic compound **17** (IC_{50}(M-CPT I) vs IC_{50}(L-CPTI) = 39.4), as well as for other ureidic or carbamic compounds. Diabetic db/db mice treated orally with **17** and **7** for 45 days at a dose of 50 mg/kg twice a day showed a good reduction of serum glucose levels with respect to the untreated db/db mice ($p < 0.01$). In addition, **17** showed antiketotic activity in normal fasted rats. **17** has been selected for development as a potential antiketotic and antidiabetic drug.

Introduction

Diabetes mellitus is the only noninfectious disease recognized as epidemic by the World Health Organization because of its worldwide diffusion, expecially in Western life-style countries. It can be divided into two major categories: type I, or insulin-dependent diabetes mellitus (IDDM), and the more common type II, or non-insulin-dependent diabetes mellitus (NIDDM), the latter accounting for more than 90% of all diabetic patients. In type II diabetes, characterized by insulin resistance and inadeguate β-cells activity, increased fatty acid oxidation contributes greatly to hyperglycemia by formation of high levels of acetyl-coenzyme A, ATP, and NADH, which increase gluconeogenesis and thus hepatic glucose production.[1,2] The mitochondrial oxidation of long-chain fatty acids requires the intervention of two membrane-bound carnitine-dependent long-chain acyltransferases, also known as carnitine palmitoyltransferases (CPT I and CPT II).[3] CPT I, the outer mitochondrial membrane enzyme present as two isoforms known as liver (L-CPT I) and muscle (M-CPT I) isoforms, catalyzes the formation of long-chain acylcarnitines. CPT II, the inner mitochondrial membrane enzyme present as a single isoform, reconverts long-chain acylcarnitines into long-chain acyl coenzyme A thioesters. A third protein, acylcarnitine translocase, is delegated to the transport across the membrane. Long-chain acyl-CoAs are then β-oxidized to acetyl-coenzyme A, which activates the key gluconeogenetic enzyme pyruvate carboxylase.[4] CPT inhibitors indirectly reduce liver gluconeogenesis by lowering the level of acetyl-coenzyme A and are hence helpful in the treatment of type II diabetes as hypoglycemic agents.[5,6]

Oxirane carboxylates, such as etomoxir and methyl 2-tetradecylglycidate,[5] are reported to be irreversible inhibitors of CPT systems, performing good activity as hypoglycemic compounds. It is worth noting that their clinical development as hypoglycemic agents was discontinued possibly because they are equally active in inhibiting both L- and M-CPT I isoforms. Side effects such as cardiac hypertrophy have also been observed.[7] Quite recently, scientists from Novartis (Sandoz at the time) have designed and evaluated carnitine derivatives as reversible inhibitors of L-CPT I, with a partial selectivity for L-CPT I versus the M-CPT I isoform (SDZ-CPI-975 in particular). This results in an improved approach for pharmacological intervention on fatty acid oxidation, with indirect glycemic control without induction of myocardial hypertrophy.[8,9]

* To whom correspondence should be addressed. Phone: (+39-06-91393612). Fax: (+39-06-91393638). E-mail: fabio.giannessi@sigma-tau.it.

† Department of Chemical Research, Sigma Tau Pharmaceutical Industries S.p.A.

‡ Department of Endocrinology & Metabolism, Sigma Tau Pharmaceutical Industries S.p.A.

§ University of L'Aquila.

|| Department of Analytical Research, Sigma Tau Pharmaceutical Industries S.p.A.

⊥ Present address: Vascular and Metabolic Disease, Pharmaceutical Division, F. Hoffmann-La Roche Ltd., CH-4070 Basel, Switzerland.

10.1021/jm020979u CCC: $25.00 © 2003 American Chemical Society
Published on Web 12/17/2002

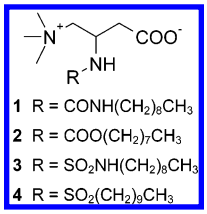
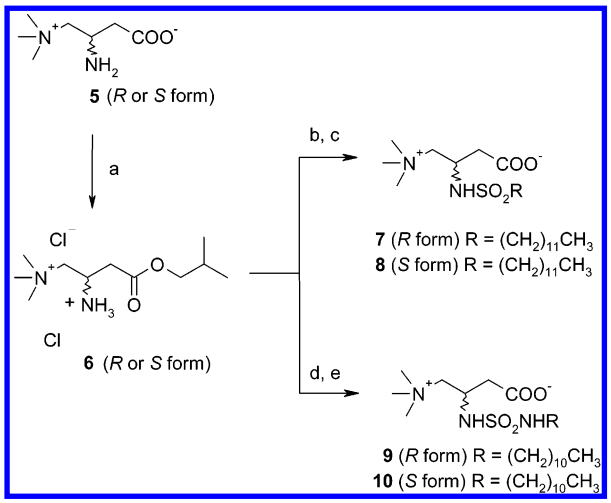


Figure 1. Racemic aminocarnitine derivatives.

Scheme 1[a]



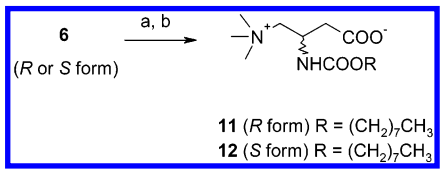
[a] (a) HCl(g) in isobutyl alcohol, 130 °C, 18 h, 95%; (b) 2.5 equiv of $CH_3(CH_2)_{11}SO_2Cl$, 5.5 equiv of NEt_3,CH_2Cl_2, 72 h; (c) NaOH 1 N, 18 h, 44% (from **6**); (d) 3 equiv of SO_2Cl_2, 4 equiv of NEt_3, CH_2Cl_2, 3 h, then 2 equiv of NEt_3, 2 equiv of $CH_3(CH_2)_{10}NH_2$,18 h; (e) NaOH 1 N, 18 h, 38% (from **6**).

In our previous work,[10] while pursuing the idea that carnitine analogues, with medium to long alkyl chains, could occupy the catalytic site of the enzyme, thus slowing down the rate of conversion of long-chain acyl-CoA into long-chain acylcarnitines,[11,12] we found that racemic aminocarnitine derivatives act as good L-CPT I inhibitors (the relative structures **1**−**4** are reported in Figure 1). The most active compounds, sulfamide **3** and sulfonamide **4**, were then synthesized in enantiomerically pure form with a slight modulation of the chain length. In particular, the optically pure (*R*)-sulfonamide **7** and (*R*)-sulfamide **9** (Scheme 1) confirmed the expected good L-CPT I inhibition in vitro. In addition, the only compound selected at that time for in vivo tests, **7**, showed hypoglycemic activity in db/db mice, a model of type II diabetes, and antiketotic activity in normal fasted rats.

Ketoacidosis, in fact, is a serious life-threatening complication especially in type I diabetes (but also in type II),[13] and it is a major source of morbidity and mortality in diabetic children and adolescents.[14] Since ketone bodies are the major products of liver fatty acid oxidation, a liver-specific CPT I inhibitor is actually expected to significantly reduce their blood levels.

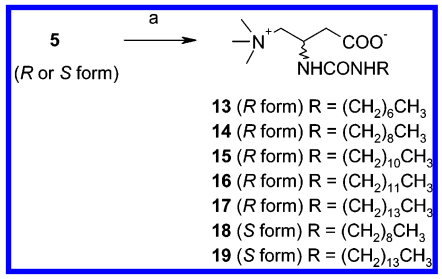
In this paper, we report the results of a more detailed study into the effects of chirality and chain length, in aminocarnitine derivatives carrying functional groups such as those reported in Figure 1, on CPT inhibition. Actually, the chain length effect was thoroughly evaluated within the ureido derivative series, while for the other compounds it was kept unchanged.[10] For selected molecules, we also report the results of in vivo experiments in diabetic mice and in fasted rats, which allowed the identification of **17**, ST 1326, the (*R*)-4-trimethylammonio-3-[(tetradecylcarbamoyl)amino]butyrate, as a candidate for preclinical and clinical development as an antiketotic and antidiabetic agent.

Scheme 2[a]



[a] (a) 2.5 equiv of $CH_3(CH_2)_7OCOCl$, 2.5 equiv of NEt_3, CH_2Cl_2, 18 h, 71%; (b) IRA 402 resin (OH^-), 94% (66% from **6**).

Scheme 3[a]



[a] (a) 2 equiv of RNCO, DMSO, 40 °C, 60 h, 47−68% (method A); or 1 equiv of RNCO, MeOH, 18 h, 52% (method B).

Chemistry

The (*R*) and (*S*) sulfonamide compounds characterized by a linear alkyl chain of 12 carbons (**7** and **8**, respectively) were prepared starting from (*R*)- or (*S*)-aminocarnitine **5**,[15] as illustrated in Scheme 1, via transformation of the corresponding enantiomeric form into its isobutyl ester **6**, followed by reaction with dodecanoyl sulfonyl chloride and final hydrolysis. In the same scheme, we also report the preparation of (*R*)- and (*S*)-sulfamide derivatives with linear alkyl chains, in this case of 11 carbons (**9** and **10**, respectively), for a safe comparison with **7** and **8**, having the same overall side chain length. Thus, **6** was treated with SO_2Cl_2 in the presence of triethylamine, followed by reaction with undecylamine and subsequent hydrolysis to the final compound.

(*R*)-Carbamic and (*S*)-carbamic derivatives with linear alkyl chains of 8 carbons (**11** and **12**, respectively) were prepared as reported in Scheme 2, starting from **6** by reaction with octylchloroformiate, followed by final hydrolysis.

(*R*)-Ureidic and (*S*)-ureidic derivatives with linear alkyl chains of 9 carbons (**14** and **18**, respectively) and 14 carbons (**17** and **19**, respectively) were prepared as reported in Scheme 3, by reaction of (*R*)- or (*S*)-aminocarnitine with the appropriate alkyl isocyanate. For ureidic compounds in the (*R*) form, a more extensive modulation of the chain length was performed, also synthesizing compounds **13** (7-carbon alkyl chain), **14** (9 carbons), **15** (11 carbons), and **16** (12 carbons) always by reaction of (*R*)-aminocarnitine with the appropriate alkyl isocyanate. When the isocyanate was not commercially available, it was prepared from the appropriate carboxylic acid via Curtius reaction, following the procedure described in the reference reported in the Experimental Section.

Table 1. Effect of CPT I Inhibitors on Mitochondrial CPT I Activity[a]

compd	chirality	chain length (no. of carbon atoms)	IC_{50} (liver) [b] (μM)	IC_{50} (heart)[b] (μM)
7	(*R*)	12	0.7	3.4
8	(*S*)	12	6	>10
9	(*R*)	11	0.8	5.8
10	(*S*)	11	6	>10
11	(*R*)	8	9.5	80.1
12	(*S*)	8	>300	>300
13	(*R*)	7	43.6	>300
14	(*R*)	9	31.3	46.2
15	(*R*)	11	3.2	118.4
16	(*R*)	12	1.8	>120
17	(*R*)	14	1.1	43.4
18	(*S*)	9	>300	>300
19	(*S*)	14	58.8	167.4
SDZ-CP1975[8,9]	(*R*)	14	17.4	62

[a] The activity of CPT I was measured radiochemically by using [^{14}C]-palmitoyl-CoA and carnitine itself by evaluating the incorporation of [^{14}C]-palmitoyl residue on carnitine in intact fresh liver and heart mithocondria from Sprague−Dawley male rats.[16,17] [b] The IC_{50} values (μM) were calculated from the inhibition curve. Data are the mean of two determinations in triplicate. The variation was less than 8%.

To assess the chemical purity of final compounds, since contamination from long-chain reagents may raise some concern, HPLC was also extensively used (see Experimental Section, general procedures). The water content of these hygroscopic molecules, measured by the Karl−Fischer method, was considered for screening purposes. Because the starting material **5** is optically pure (see ref 15), enantiomeric excess was checked only in the case of one derivative, namely, compound **17**. As expected, when chiral HPLC (Chirobiotic-Tag column, 5 μm, 250 × 4.6 mm) was used, >99% ee was found.

Biology and Discussion

IC_{50} (μM) values for L-CPT I and M-CPT I, determined respectively in rat liver and heart mitochondria,[16,17] are reported in Table 1. It should be noted that the M-CPT I isoform is expressed in both cardiac and skeletal muscles. For all the functional groups, the highest activity in vitro was observed for compounds in the (*R*) form, thus confirming our early results in the case of sulfonamide and sulfamide compounds,[10] as well as what was already reported for the Sandoz compound SDZ-CPI-975, which we used as a reference.[9]

The importance of the chain length was demonstrated in the ureidic series by testing the (*R*) compounds carrying 7 (**13**), 9 (**14**), 11 (**15**), 12 (**16**), and 14 (**17**) term chains, the longer alkyl chain showing the highest activity. In this series, the IC_{50} values ranged from 1.1 μM for **17** to 43.6 μM for compound **13**. For SDZ-CPI-975, the IC_{50} was 17.4 μM. In addition, even if L-CPT I inhibition was quite similar for ureido **17**, sulfonamide **7**, and sulfamide **9**, an unexpected extraordinarily high selectivity for liver with respect to muscle isoform was observed for **17** (40-fold selectivity against a 4-fold selectivity for SDZ-CPI-975). It is also worth emphasizing that such a selectivity is reported as particularly important in order to avoid undesirable side effects on the cardiac muscle, leading to heart hypertrophy.[7] In any case, also for **7** and **9**, as well as for the other ureido and carbamate compounds, a good selectivity was observed.

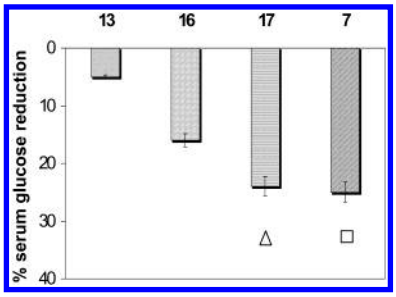


Figure 2. Percent reduction of serum glucose after 45-day treatment in db/db mice via oral gavage, at doses equivalent to 50 mg/kg of **17** twice a day, determined after 9 h of fasting and 9 h from last treatment. Percent reduction is calculated with respect to untreated db/db mice used as control. The number of animals per group is 8. Student's *t* test indicate (□) $p < 0.05$ and (△) $p < 0.01$ versus control diabetic mice.

All the compounds were tested for their aspecific toxicity on cultures of rat hepatocytes by measuring the release of LDH (lactate dehydrogenase) after 48 h of incubation.[18] No toxicity was observed at concentrations up to 50 μM.

The ureido derivatives **13**, **16**, and **17**, together with the sulfonamide compound **7**, were then selected for the in vivo studies in db/db mice (C57BL/KsJ-db/db), a model of type II diabetes,[19] and were tested at oral doses equimolar to 50 mg/kg, referenced to **17**, twice a day for 45 days ($N = 8$ for each group). In particular, **7** and **17** showed a good reduction of serum glucose levels with respect to untreated db/db mice (Figure 2, −25%; from 673.9 ± 44.09 to 506.6 ± 59.12 mg/dL for **17**; in lean mice, serum glucose level was 202.6 ± 5.91 mg/dL; mean ± SE; $p < 0.01$). For compounds of the ureido series, a decreasing activity in vivo, parallel to the decrease in alkyl chain length, was observed as expected on the basis of the in vitro L-CPT I inhibition. Treatment with **17** showed no significant effects on food intake and body weight, whereas water consumption was reduced (−30%), and a strong improvement in diabetic polyuria was observed. The reduction of glucose was also accompanied by a significant decrease of serum fructosamine levels, a measure of total glucose exposure (from 4.80 ± 0.17 to 3.36 ± 0.30 mmol/L, −30%; in lean mice, serum fructosamine level was 3.37 ± 0.16 mmol/L; mean ± SE; $p < 0.01$). Liver glycogen was also significantly reduced (−25%), while triglycerides (TG) content was significantly increased (+38%). Serum insulin levels were not significantly modified nor were leptin, TG, urea, alanine aminotransferase, and cholesterol levels; free fatty acid (FFAs) levels were instead significantly increased (+20%). It is known that an increase of serum FFAs is correlated to a deterioration of insulin resistance,[20] but our results indicate that long-term treatment with **17** seems to improve glucose homeostasis in db/db mice. In any case, further investigation into different experimental models will be undertaken to better characterize compound **17**.

In treated mice, no effects on heart weight and TG content were observed, thus confirming the preferential inhibition of hepatic CPT I activity and therefore its predictable safety with regard to cardiac hypertrophy.

Furthermore, a kinetic study revealed that compound **17** is a reversible, mixed inhibitor of L-CPT I with respect to palmitoyl-CoA (apparent $K_i = 0.36 \pm 0.04$ μM).

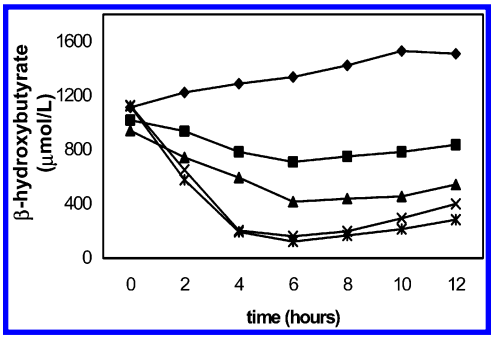


Figure 3. Levels of β-hydroxybutyrate (μmol/L) in 24 h fasted rats at 2, 4, 6, 8, 10, and 12 h after treatment with compound **17** at oral doses of 0 (◆) 10 (■), 20 (▲), 30 (×), and 40 (∗) mg/kg. Values are the mean ± SE of six animals per group.

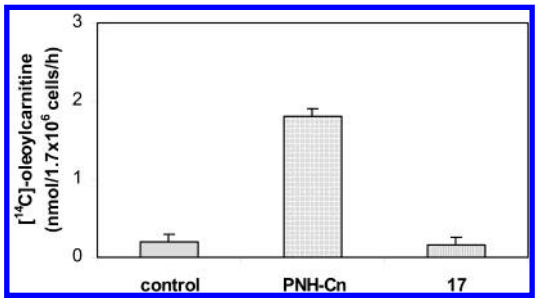


Figure 4. [^{14}C]-oleoylcarnitine level, as indicator of CPT II inhibition, after rat hepatocytes incubation with **17** (3 mM) or (*R*)-*N*-palmitoylaminocarnitine (PNH-Cn, 1 mM).

To confirm the inhibition of liver CPT I in an additional animal model and to verify its antiketotic effect, **17** was orally administered to 24 h fasted normal rats. Since β-hydroxybutyrate (β-HBA) is a major product of liver fatty acid oxidation during fasting,[21] a CPT I inhibitor is expected to significantly reduce its level. We actually observed a strong dose-dependent reduction of serum β-hydroxybutyrate levels up to12 h from acute oral dose, with ED_{50} = 14.5 mg/kg (Figure 3). The rate of production of β-hydroxybutyrate seems to increase after 6−8 h, indicating the recovery of liver β-oxidation. When **19** ((*S*)-form) was administered, no effect on β-HBA levels was observed, again confirming the analogy between the results of the in vitro and the in vivo tests and excluding any hypothetical aspecific effect due to the structure of the molecule.

Because inhibition of CPT II, the CPT isoform common to all tissues, may at least in part nullify efforts to obtain a selective liver-specific inhibitor, we also tested the ability of oleate-treated hepatocytes, preincubated with compound **17**, to transform oleoylcarnitine (originating from oleate itself) again into oleoyl-CoA. Higher levels of oleoylcarnitine in treated hepatocytes with respect to control values are therefore indicators of the inhibition of CPT II activity. Compound **17** (3 mM) did not show any variation in oleoylcarnitine levels with respect to control values (Figure 4), while the known closely related CPT inhibitor (*R*)-*N*-palmitoylaminocarnitine[22] (1 mM) showed about a 10-fold increment, thus showing a strong CPT II inhibiting activity.

In a preliminary stability test, at pH values simulating those of stomach and gut conditions (pH 2.0 and 8.5, respectively), **17** showed complete stability. Once again, the closely related (*R*)-*N*-palmitoylaminocarnitine behaved differently, with its concentration (determined by HPLC) beginning to decrease under both conditions just after 2 h.

In conclusion, in this work we reported the identification of compound **17** as an agent able to selectively inhibit in vitro L-CPT I with respect to M-CPT I and to depress ketogenesis in vivo (fasted rats). **17** also showed a good reduction of serum glucose levels in a diabetic mouse model, without any significant change in heart weight and TG content. Owing to its encouraging activity and pharmacological profile, **17** was selected as a candidate for clinical development as an antiketotic and antidiabetic drug. Nevertheless, since it has been reported that development of the aforementioned Novartis compound, SDZ-CPI-975, was stopped owing to "hepatic mitochondrial aberrations",[6] this will be considered during the investigations into the safety of our compound, before committing to full development.

Experimental Section

Chemistry. General Procedures. Melting points were determined in open capillary tubes and are uncorrected.

^{1}H NMR spectra were recorded on a VXR 300S Varian spectrometer at 300 MHz or on a Gemini 200 Varian at 200 MHz. The chemical shift δ is expressed in parts per million relative to the standard tetramethylsilane. Ion spray mass spectra were recorded in positive mode on an ESI LCQ Classic Thermo-Finnigan ion trap spectrometer. Flash column chromatography separations were carried out with Biotage Flash 40i apparatus using KP-Sil silica cartridge (32−63 μm, 60 Å, 500−550 m^2/g silica). Thin-layer chromatography (TLC) was performed on silica gel (5 cm × 10 cm) 60 F254 Merck prescored plates. Reagents and solvents were purchased from common suppliers and were used as received. All starting materials were commercially available with the exception of (*R*)- and (*S*)-aminocarnitine, together with nonylisocyanate, which were prepared according to literature procedures (see ref 15 for aminocarnitine; see *Org. Synth.* **1967**, (Collective) *3*, 846−847 for the synthetic procedure described in the case of undecylisocyanate and used for nonylisocyanate).

General conditions for HPLC of the final compounds are the following: column = Spherisorb S5 SCX, 5 μm, 250 mm × 4.6 mm, mobile phase = 50 mM $(NH_4)H_2PO_4/CH_3CN$ 60/40 (v/v), pH 3.7 with H_3PO_4, T = 30 °C, flow = 0.75 mL/min, IR and UV detectors); or Spherisorb-C1, 5 μm, 250 mm × 4.6 mm, mobile phase = 50 mM KH_2PO_4/CH_3CN 60/40 (v/v), pH = as it was, T = 30 °C, flow = 0.75 mL/min, RI and UV detectors). To exclude contamination from long-chain impurities, the following was used: symmetry C_{18} 3 μm, 75 mm × 4.6 mm, mobile phase = CH_3CN/H_2O 80/20 (v/v), pH 3.7 with H_3PO_4, T = 25 °C, flow = 1.50 mL/min, RI and UV detectors).

(*R*)-Aminocarnitine Isobutyl Ester Chloride Hydrochloride ((*R*)-6, (Isobutyl (*R*)-4-Trimethylammonio-3-aminobutyrate Chloride Hydrochloride). A suspension of (*R*)-aminocarnitine, (*R*)-**5** (3.0 g, 18.72 mmol), in isobutyl alcohol (120 mL) at 4 °C (ice bath) was saturated with gaseous HCl, and the resulting solution was refluxed for 18 h (oil bath temperature = 130 °C). The solvent was evaporated under vacuum, and the residue was triturated with ethyl ether to yield the product as a white solid (5.1 g, 95% yield): mp 115−116 °C (dec); $[\alpha]^{20}_D$ + 6.8° (*c* 0.5, MeOH); ^{1}H NMR (200 MHz, D_2O) δ 4.35 (m, 1H), 4.00 (d, 2H), 3.88 (d, 2H), 3.28 (s, 9H), 3.10 (m, 2H), 1.95 (m, 1H), 0.90 (d, 6H); KF = 1.0%. Anal. ($C_{11}H_{26}Cl_2N_2O_2$), C, H, N.

Alternatively, $SOCl_2$ (molar ratio 6:1) was used instead of gaseous HCl and the amount of isobutyl alcohol was reduced (600 mL for 10 g of aminocarnitine). The residue obtained after trituration with ethyl ether was also triturated with CH_2Cl_2 to give (*R*)-**6** (96% yield).

(*S*)-Aminocarnitine Isobutyl Ester Chloride Hydrochloride ((*S*)-6). The compound was prepared as described

in the case of (*R*)-**6**, but starting from (*S*)-aminocarnitine (*S*)-**5**: mp 116−117 °C (dec); $[\alpha]^{20}_D$ − 6.9° (*c* 0.5, MeOH); KF = 0.8%. Anal. ($C_{11}H_{26}Cl_2N_2O_2$), C, H, N.

(*R*)-4-Trimethylammonio-3-[(dodecanesulfonyl)amino]-butyrate (7). NEt_3 (2.65 mL, 1.92 g, 19 mmol) and dodecanesulfonyl chloride (2.33 g, 8.65 mmol, in 3 mL of dry CH_2Cl_2) were added to a solution of (*R*)-aminocarnitine isobutyl ester chloride hydrochloride (*R*)-**6** (1.0 g, 3.46 mmol) in dry CH_2Cl_2 (5 mL) at 0 °C. The mixture was left under stirring for 72 h at room temperature. The solvent was evaporated under vacuum, the residue was taken up in EtOAc (100 mL), and the white precipitate (NEt_3·HCl) thus obtained was filtered off under vacuum. The EtOAc solution was evaporated under vacuum to give 2.8 g of yellow oil; 1 N NaOH (71 mL) was then added to the oil in order to perform ester hydrolysis, leaving the suspension under stirring overnight. After evaporation under vacuum to dryness, the crude product was purified by flash chromatography using MeOH as eluant to yield 597 mg of **7** (44% yield): mp 157−158 °C (dec); $[\alpha]^{20}_D$ −7° (*c* 0.265, H_2O); ^{1}H NMR (CD_3OD, 300 MHz) δ 4.30 (m, 1H), 3.45 (m, 2H), 3.28 (s, 9H), 3.15 (m, 2H), 2.45 (d, 2H), 1.78 (m, 2H), 1.45 (m, 2H), 1.30 (brs, 16H), 0.90 (brt, 3H); TLC, eluant $CHCl_3$/*i*PrOH/MeOH/H_2O/CH_3COOH 42:7:28:10.5:10.5, R_f = 0.6; MS (ES) *m*/*z* 393 (MH^+); KF 6.9%. Anal. ($C_{19}H_{40}N_2O_4S$) C, H, N.

(*S*)-4-Trimethylammonio-3-[(dodecanesulfonyl)amino]-butyrate (8). The product was prepared as described in the case of (*R*)-**7**, but starting from (*S*)-**6**: mp 156−157 °C (dec); $[\alpha]^{20}_D$ +6° (*c* 0.245, H_2O); KF 8.6%. Anal. ($C_{19}H_{40}N_2O_4S$) C, H, N.

(*R*)-4-Trimethylammonio-3-[(undecylsulfamoyl)amino]-butyrate (9). A solution of SO_2Cl_2 (1.7 mL, 2.86 g, 21.16 mmol) in dry CH_2Cl_2) (10 mL) was added dropwise to a solution of (*R*)-aminocarnitine isobutylester chloride hydrochloride (*R*)-**6** (2 g, 6.9 mmol) and NEt_3 (3.84 mL, 2.79 g, 27.6 mmol) in dry CH_2Cl_2 (40 mL) at 0°C. The mixture was left 3 h under stirring at room temperature. Then, additional NEt_3 (1.92 mL, 1.39 g, 13.8 mmol) and undecylamine (2.97 mL, 2.36 g, 13.8 mmol) were added, and the mixture was left overnight under stirring at room temperature. The solvent was evaporated under vacuum, the residue was taken up with EtOAc (100 mL), and the precipitate (NEt_3·HCl) was filtered off under vacuum. The EtOAc solution was evaporated under vacuum to yield 4.8 g of yellow oil: 1 N NaOH (105 mL) was then added to the oil in order to perform ester hydrolysis, leaving the suspension under stirring overnight at room temperature. After evaporation under vacuum to dryness, the crude yellow product was purified by flash chromatography using $CHCl_3$/MeOH (from 9/1 to 1/9) as eluant. The product thus obtained was further purified by flash chromatography using MeOH as eluant, to yield, after solvent evaporation under vacuum, compound **9** in 38% yield: mp 157−159 °C (dec); $[\alpha]^{20}_D$ −4° (*c* 0.25, H_2O, pH 2 with HCl); ^{1}H NMR (CD_3OD, 300 MHz) δ 4.10 (m, 1H), 3.45 (d, 2H), 3.25 (s, 9H), 2.95 (m, 2H), 2.50 (m, 2H), 1.55 (brm, 2H), 1.35 (brs, 16H), 0.90 (brt, 3H); TLC, silica gel, $CHCl_3$/*i*PrOH/MeOH/H_2O/CH_3COOH 42:7:28:10.5:10.5, R_f = 0.7; MS (ES) *m*/*z* 394 (MH^+); KF 4.7%. Anal. ($C_{18}H_{39}N_3O_4S$) C, H, N.

(*S*)-4-Trimethylammonio-3-[(undecylsulfamoyl)amino]-butyrate (10). The product was prepared as described in the case of (*R*)-**9**, but starting from (*S*)-**6**: mp 152−154 °C (dec); $[\alpha]^{20}_D$ +4° (*c* 0.25%, H_2O, pH 2 with HCl); KF 2.9%. Anal. ($C_{18}H_{39}N_3O_4S$) C, H, N.

(*R*)-4-Trimethylammonio-3-[(octyloxycarbonyl)amino]-butyrate (11). Octyl chloroformate (2.52 g, 13 mmol) and NEt_3 (1.8 mL, 1.31 g, 13 mmmol) were added to a solution of (*R*)-aminocarnitine isobutyl ester chloride hydrochloride (*R*)-**6** (1.5 g, 5.2 mmol) in anhydrous CH_2Cl_2 (30 mL). The reaction was left 18 h under stirring at room temperature, the solvent was evaporated under vacuum, and the residue was dissolved in EtOAc and filtered. The filtrate was evaporated under vacuum to give a residue that was purified by silica gel flash chromatography, using a $CHCl_3$ to $CHCl_3$/CH_3OH 9:1 gradient to give 1.5 g (71% yield) of the intermediate ester compound: mp 80−82 °C, $[\alpha]^{20}_D$ −15.2° (*c* 0.45, H_2O); ^{1}H NMR (CD_3OD, 300 MHz) δ 4.56−4.46 (m, 1H), 4.12−4.02 (m, 2H), 3.94−3.88 (m, 2H), 3.66−3.50 (m, 2H), 3.20 (s, 9H), 2.74−2.66 (m, 2H), 2.05−1.86 (m, 1H), 1.68−1.56 (m, 2H), 1.45−1.25 (m, 10H), 1.05−0.85 (m, 9H). This intermediate was transferred into an Amberlite IRA-402 column (OH^- form) and eluted with water. The water was evaporated under vacuum, and the residue was triturated with acetone and filtered to give compound **11** as a white solid in 94% yield (66% yield from **6**): mp 155−156 °C (dec); $[\alpha]^{20}_D$ −14.2° (*c* 0.5, H_2O); ^{1}H NMR (CD_3OD, 300 MHz) δ 4.40 (m, 1H), 4.05 (t, 2H), 3.52 (d, 2H), 3.20 (s, 9H), 2.42 (d, 2H), 1.60 (m, 2H), 1.42−1.22 (m, 10H), 0.90 (brt, 3H); TLC, silica gel, $CHCl_3$/*i*PrOH/MeOH/H_2O/CH_3COOH 42:7:28:10.5:10.5; R_f = 0.6; MS (ES) *m*/*z* 317 (MH^+); KF = 5.7%. Anal. ($C_{16}H_{32}N_2O_4$) C, H, N.

(*S*)-4-Trimethylammonio-3-[(octyloxycarbonyl)amino]-butyrate (12). The product was prepared as described in the case of (*R*)-**11**, but starting from (*S*)-**6**: mp = 157−158 °C (dec); $[\alpha]^{20}_D$ +13.6° (*c* 0.46, H_2O); KF = 2.1%. Anal. ($C_{16}H_{32}N_2O_4$) C, H, N.

(*R*)-4-Trimethylammonio-3-[(tetradecylcarbamoyl)-amino]butyrate (17). Method A. Tetradecyl isocyanate (25.10 mL, 21.81 g, 91.12 mmol) was added to a mixture of (*R*)-aminocarnitine (*R*)-**5** (7.30 g, 45.56 mmol) in anhydrous DMSO (350 mL), and the reaction mixture was left for 60 h at 40 °C. The mixture was transferred into an Erlenmeyer flask (3 L) containing ethyl ether (2.5 L), and the solvent was separated by decanting. The residue was dissolved in $CHCl_3$, transferred into a flask, and precipitated again with ethyl ether. The crude product thus obtained was washed several times with ethyl ether and purified by silica gel chromatography, using a MeOH/$CHCl_3$ 1:9 to MeOH/$CHCl_3$ 8:2 gradient until elution of impurities with higher R_f, then eluting the product with MeOH/$CHCl_3$ 9:1. Compound **17** was obtained as a white solid (10.38 g, 57% yield): mp 160−162 °C (dec); $[\alpha]^{20}_D$ −21.1° (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD, 300 MHz) δ 4.52 (m, 1H), 3.60 (dd, 1H), 3.48 (d, 1H), 3.20 (s, 9H), 3.10 (t, 2H), 2.40 (m, 2H), 1.45 (m, 2H), 1.28 (brs, 22H), 0.90 (brt, 3H); TLC, silica gel, $CHCl_3$/*i*PrOH/MeOH/H_2O/CH_3COOH 42:7:28:10.5:10.5; R_f = 0.5; MS (ES) *m*/*z* 400 (MH^+); KF = 2.5%. Anal. ($C_{22}H_{45}N_3O_3$) C, H, N.

Method B. To a solution of (*R*)-aminocarnitine (*R*)-**5** (22.0 g, 137.5 mmol) in MeOH (120 mL), tetrabutylammonium chloride (200 mg) and, slowly, tetradecylisocyanate (44 mL, 38.23 g, 160 mmol) were added under stirring, keeping the temperature at 5°C (tetradecyl isocyanate was added in about 1.5 h; once the first 10 mL was added, the solution became a suspension). The suspension was stirred at 5 °C for one night, and additional MeOH (120 mL) was added. The suspension was filtered, and the filtrate was evaporated under vacuum. Acetone (100 mL) was added to the resulting oil to yield a cream-colored solid. After one night, the solid was separated by filtration, washed with acetone, and dried under vacuum. About 40 g of product **17** thus obtained were dissolved in MeOH (73 mL) and filtered on Celite. The filtrate was transferred into a flask containing acetone (690 mL) under stirring, giving at first a suspension that at the end of the operation became a clear solution. After 3 days at 5 °C, 28.8 g of a light cream-colored solid was obtained (yield = 52%; additional crops, usually slightly less pure, can be obtained from the liquor by standing at 5°C).

Alternatively, the crude product obtained by evaporation of the reaction solvent (MeOH) was purified by chromatography as described in method A.

(*R*)-4-Trimethylammonio-3-[(heptylcarbamoyl)amino]-butyrate (13). The compound was prepared as described in the case of **17** (method A), starting from heptyl isocyanate, and was further purified after chromatography by crystallization (CH_3CN), giving a 47% yield: mp 149−150 °C (dec); $[\alpha]^{20}_D$ −34.0° (*c* 0.97, MeOH); ^{1}H NMR (CD_3OD, 300 MHz) δ 4.52 (m, 1H), 3.60 (dd, 1H), 3.48 (d, 1H), 3.20 (s, 9H), 3.10 (t, 2H), 2.40 (m, 2H), 1.45 (m, 2H), 1.30 (brs, 8H), 0.90 (brt, 3H); TLC, silica gel, $CHCl_3$/*i*PrOH/MeOH/H_2O/CH_3COOH 42:7:28:10.5:10.5, R_f = 0.5; MS (ES) *m*/*z* 302 (MH^+); KF = 6.2%. Anal. ($C_{15}H_{31}N_3O_3$) C, H, N.

(*R*)-4-Trimethylammonio-3-[(nonylcarbamoyl)amino]-butyrate (14). The compound was prepared as described in the case of **17** (method A), starting from nonyl isocyanate, giving a 68% yield: mp 145−147 °C (dec); $[\alpha]^{20}_D$ −15.4° (*c* 0.5, H_2O); ^{1}H NMR (D_2O, 300 MHz) δ 4.52 (m, 1H), 3.60 (dd, 1H), 3.45 (d, 1H), 3.18 (s, 9H), 3.10 (t, 2H), 2.40 (d, 2H), 1.45 (m, 2H), 1.28 (brs, 12H), 0.90 (brt, 3H); TLC, silica gel, $CHCl_3$/*i*PrOH/MeOH/H_2O/CH_3COOH 42:7:28:10.5:10.5, R_f = 0.5; MS (ES) *m*/*z* 330 (MH^+); KF = 2.8%. Anal. ($C_{17}H_{35}N_3O_3$) C, H, N.

(*R*)-4-Trimethylammonio-3-[(undecylcarbamoyl)amino]butyrate (15). The compound was prepared as described in the case of **17** (method A), starting from undecyl isocyanate, and further purified after chromatography by crystallization (CH_3CN), giving a 50% yield: mp 150−151 °C (dec); $[\alpha]^{20}_D$ −21.2° (*c* 1, MeOH); ^{1}H NMR (CD_3OD, 300 MHz) δ 4.52 (m, 1H), 3.60 (dd, 1H), 3.48 (d, 1H), 3.20 (s, 9H), 3.10 (t, 2H), 2.40 (m, 2H), 1.45 (m, 2H), 1.28 (brs, 16H), 0.90 (brt, 3H); TLC, silica gel, $CHCl_3$/*i*PrOH/MeOH/H_2O/CH_3COOH 42:7:28:10.5:10.5, R_f = 0.5; MS (ES) *m*/*z* 358 (MH^+); KF = 2.3%. Anal. ($C_{19}H_{39}N_3O_3$) C, H, N.

(*R*)-4-Trimethylammonio-3-[(dodecylcarbamoyl)amino]butyrate (16). The compound was prepared as described in the case of **17** (method A), starting from dodecyl isocyanate, giving a 55% yield: mp 147−149 °C (dec); $[\alpha]^{20}_D$ −24.6° (*c* 0.48, MeOH); ^{1}H NMR (CD_3OD, 300 MHz) δ 4.52 (m, 1H), 3.60 (dd, 1H), 3.48 (d, 1H), 3.20 (s, 9H), 3.10 (t, 2H), 2.40 (m, 2H), 1.45 (m, 2H), 1.28 (brs, 18H), 0.90 (brt, 3H); TLC, silica gel, $CHCl_3$/*i*PrOH/MeOH/H_2O/CH_3COOH 42:7:28:10.5:10.5, R_f = 0.6; MS (ES) *m*/*z* 372 (MH^+); KF = 5.4%. Anal. ($C_{20}H_{41}N_3O_3$) C, H, N.

(*S*)-4-Trimethylammonio-3-[(nonylcarbamoyl)amino]-butyrate (18). The compound was prepared as described in the case of **17** (method A), starting from nonyl isocyanate and (*S*)-aminocarnitine: mp 146−147 °C (dec); $[\alpha]^{20}_D$ +16.7° (*c* 0.43, H_2O); KF = 1.8%. Anal. ($C_{17}H_{35}N_3O_3$) C, H, N.

(*S*)-4-Trimethylammonio-3-[(tetradecylcarbamoyl)amino]butyrate (19). The compound was prepared as described in the case of **17** (Method A), starting from tetradecyl isocyanate; mp 166−167 °C (dec); $[\alpha]^{20}_D$ +20.7° (*c* 0.5, MeOH); KF = 1.7%. Anal. ($C_{22}H_{45}N_3O_3$) C, H, N.

Pharmacology. Determination of CPT I Activity Inhibition in Vitro. CPT I inhibition was evaluated with minor modifications as described in ref 17 on fresh intact liver or heart mitochondrial preparations obtained from normally fed Sprague−Dawley male rats (provided by Charles River Italia).[16] Mitochondria were isolated from liver or heart and suspended in a buffer containing 75 mM (for heart) or 225 mM (for liver) sucrose, 1 mM EGTA (pH 7.5), mannitol 225 mM, and 5 mM Tris-HCl. The assay medium (100 μL) containing 120 mM KCl, 1 mM EGTA (pH 7.5), 10 mM HEPES (pH 7.5), 20 mM sucrose, 1 mM DTT, 8.3 μM BSA, 50 mM [^{14}C]-palmitoyl-CoA (specific activity 10000 DPM/mole), and 10 mM L-carnitine was incubated at 37 °C in the presence of scalar concentrations of the test compound (0−3 mM). Reaction time 1 min. Data are the mean of two determinations in triplicate. The variation between experiments was less than 8%.

Antidiabetic Activity in Vivo. Male C57BL/KsJ-db/db mice were obtained at 6 weeks of age from Jackson Laboratories and maintained at 25 ± 2 °C on a 12 h light−dark cycle. Animals were given standard laboratory food and water, ad libitum, and were used for experiments at 8 weeks of age. Eight animals were used in each treatment group, and test compounds were administered orally at doses equimolar to **17** (50 mg/kg), twice a day for 45 days. The control animals were given vehicle carboxymethylcellulose (0.5% in deionized H_2O, dose of 10 mL/kg). At sacrifice, blood samples were collected after 9 h from the last treatment (animals in postabsorptive state fasted for 9 h). The glucose serum levels were determined in a Cobas Mira S (Roche) autoanalyzer using commercial kits (Roche), and the percent reduction values with respect to untreated control db/db mice were calculated as follows:

$$\frac{(\text{serum glucose in treated mice}) - (\text{serum glucose in control mice})}{\text{serum glucose in control mice}} \times 100.$$

Determination of β-Hydroxybutyrate Levels in Serum of Fasted Rats Treated with CPT I Inhibitors in Vivo. Normally fed Sprague−Dawley male rats (provided by Charles River Italia) (200−225 g) maintained at 22 ± 2 °C on a 12 h light−dark cycle fasted for 24 h and subsequently treated orally with **17** (10, 20, 30, and 40 mg/kg). At 0, 2, 4, 6, 8, 10, 12 h after treatment, blood was collected from the tail vein and serum was obtained after centrifugation. β-Hydroxybutyrate concentrations were determined by commercial kits (Sigma) in a Cobas Mira (Roche) autoanalyzer. Results are expressed as the mean ± SE of six animals per group.

Evaluation of CPT II Activity Inhibition in Vitro. Hepatocytes were isolated from 24 h fasted Sprague−Dawley male rats (225−250 g), provided by Harlan Italia. Liver cells were incubated for 30 min at 37 °C in 0.7 mL of gassed (95:5 O_2/CO_2) Krebs medium containing 2% defatted bovine serum albumin (BSA), 1 mM glucose, 1 mM pyruvate, 9 mM lactate, 100 mM [^{14}C]-L-carnitine (125700 dpm/nmol), and **17** (3 mM) or (*R*)-*N*-palmitoylaminocarnitine (1 mM). At these concentrations, **17** and (*R*)-*N*-palmitoylaminocarnitine markedly decreased ketone body production. After 30 min of preincubation with test compounds, 1 mM sodium oleate was added to the incubation mixture, and 2 h later the incubation was stopped with 5% $HClO_4$. The pelleted sample, containing long-chain acylcarnitine (oleoyl-[^{14}C]-L-carnitine), was washed twice with 5% $HClO_4$ and was dissolved in 1 N KOH, and then the [^{14}C]-L-carnitine radioactivity was measured. Values are the mean ± SE for three determinations done in duplicate.

Determination of LDH Levels in Vitro. Levels of LDH were evaluated using the method described in ref 18 by commercial kits with a Cobas Mira S autoanalyzer after incubation for 48 h of test compounds at different concentrations (10, 50, 100 μM) on hepatocytes obtained from Wistar male rats liver. These last were isolated and prepared for experiment as described in ref 23.

***K*$_i$ Assay Determination on CPT I Expressed in *Pichia pastoris*.** K_i and K_m values were determined by measuring L-CPT 1 activity radiochemically at different concentrations of palmitoyl-CoA (0−200 μmol/L) and **17** (0.5 and 1.0 μmol/L) and at fixed [^{14}C]-L-carnitine (500 μmol/L) concentrations[24] on yeast extract expressing L-CPT 1, obtained as described in ref 25. The [^{14}C]-palmitoylcarnitine formed was measured. Specific activity of CPT 1 was expressed as nmol min^{-1} mg^{-1} of protein. Data were fitted according to the Michaelis−Menten equation to obtain K_m or according to the equation for mixed inhibition of **17** at two concentrations (0.5 and 1.0 μmol/L) to obtain K_i. Curve fitting was performed using GraphPad Prisma software with nonlinear regression analysis and Excel software using linear regression analysis. K_i values were obtained by plotting inhibitor concentrations vs K_{mapp}/V_{maxapp} ratio.

Data are expressed as mean ± standard deviation, and the correlation coefficient (r^2) was used to evaluate the quality of the fitting.

Acknowledgment. The authors thank Giuseppina Calvisi, Enrico De Fusco, Emanuele Pani, Alessandro Peschechera, Federico Serafini, and Ornella Petrella for technical assistence. They also thank Drs. Enrico Ammannati (Argus Chemicals) and Angelo De Luca for their contribution to the chemical development of (*R*)-**5** and **17**, and Ms. Marlene Deutsch for her copyreading skills.

Supporting Information Available: ^{1}H NMR spectra for final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) DeFronzo, R. A.; Bonadonna, R. C.; Ferranini, E. Pathogenesis of NIDDM: a balanced overview. *Diabetes Care* **1992**, *15*, 318−368.

(2) Golay, A.; Swislocki, A. L. M.; Chen, Y. D.; Reaven, G. M. Relationships between plasma-free fatty acid concentration, endogenous glucose production, and fasting hyperglycemia in normal and non-insulin-dependent diabetic individuals. *Metabolism* **1987**, *36*, 692−696.

(3) McGarry, J. D.; Brown, N. F. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. *Eur. J. Biochem.* **1997**, *244*, 1−14.
(4) Attwood, P. A.; Keech, D. B. Pyruvate carboxylase. *Curr. Top. Cell. Regul.* **1984**, *23*, 1−55.
(5) Foley, J. E. Rationale and Application of Fatty Acid Oxidation Inhibitors in Treatment of Diabetes Mellitus. *Diabetes Care* **1992**, *15*, 773−784.
(6) Anderson, R. Carnitine Palmitoyltransferase: A Viable Target for the Treatment of NIDDM? *Curr. Pharm. Des.* **1998**, *4*, 1−15.
(7) Bressler, R.; Gay, R.; Copeland, J. G.; Bahl, J. J.; Bedotto, J.; Goldman, S. Chronic inhibition of fatty acid oxidation: New model of diastolic dysfunction. *Life Sci.* **1989**, *44*, 1897−1906.
(8) Deems, R. O.; Anderson, R. C.; Foley, J. E. Hypoglycemic effects of a novel fatty acid oxidation inhibitor in rats and monkeys. *Am. J. Physiol.* **1998**, *274*, R524−R528.
(9) Anderson, R. C.; Balestra, M.; Bell, P. A.; Deems, R. O.; Fillers, W. S.; Foley, J. E.; Fraser, J. D.; Mann, W. R.; Rudin, M.; Villhauer, E. B. Antidiabetic Agents: A New Class of Reversible Carnitine Palmitoyltranferase I Inhibitors. *J. Med. Chem.* **1995**, *38*, 3448−3450.
(10) Giannessi, F.; Chiodi, P.; Marzi, M.; Minetti, P.; Pessotto, P.; De Angelis, F.; Tassoni, E.; Conti, R.; Giorgi, F.; Mabilia, M.; Dell'Uomo, N.; Muck, S.; Tinti, M. O.; Carminati, P.; Arduini, A. Reversible Carnitine Palmitoyltransferase Inhibitors with Broad Chemical Diversity as Potential Antidiabetic Agents. *J. Med. Chem.* **2001**, *44*, 2383−2386.
(11) Jenkins, D. L.; Griffith, O. W. Antiketogenic and Hypoglycemic Effects of Aminocarnitine and Acylaminocarnitines. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 290−294.
(12) Savle, P. S.; Pande, S. V.; Lee, T. S.; Gandour, R. D. Stereoisomeric Acylamidomorpholinium Carnitine Analogues: Selective Inhibitors of Carnitine Palmitoyltransferase I and II. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3099−3102.
(13) Carrol, M. F.; Schade, D. S. Ten Pivotal Questions about Diabetic Ketoacidosis. *Postgrad. Med.* **2001**, *110* (5), 89−95.
(14) Felner, E. I.; White, P. C. Improving Management of Diabetic Ketoacidosis in Children. *Pediatrics* **2001**, *108* (3), 735−740.
(15) Castagnani, R.; De Angelis, F.; De Fusco, E.; Giannessi, F.; Misiti, D.; Meloni, D.; Tinti, M. O. Stereospecific Synthesis of (*R*)-Aminocarnitine (Emeriamine) Starting from (*R*)-Carnitine via Double Inversion of Configuration. *J. Org. Chem.* **1995**, *60*, 8318−8319.
(16) (a) Kerner, J.; Bieber, L. Isolation of a malonyl-CoA sensitive CPT/*β*-oxidation enzyme complex from heart mitochondria. *Biochemistry* **1990**, *29*, 4326−4334. (b) Hovius, R.; Lambrescts, H.; Nicolay, K.; De Kruijff, B. Improved methods to isolate and subfractionate rat liver mitochondria. Lipid composition of the inner and outer membrane. *Biochim. Biophys. Acta* **1990**, *1021*, 217−226.
(17) Fiol, C. J.; Kerner, J.; Bieber, L. L. Effect of malonyl-CoA on the kinetics and substrate cooperativity of membrane-bound carnitine palmitoyltransferase of rat heart mitochondria. *Biochim Biophys. Acta* **1987**, *916* (3), 482−492.
(18) Vassault, A. UV-method with pyruvate and NADH. In *Methods of Enzymatic Analysis*, 3rd ed.; Bergmeyer, H. U., Ed.; Verlag Chemie: Weinheim, Germany, 1983; Vol. 3, pp 118−126.
(19) Kodama, H.; Fujita, M.; Yamaguchi, I. Development of hyperglycaemia and insulin resistance in conscious genetically diabetic (C57BL/KsJ db/db) mice. *Diabetologia* **1994**, *37*, 739−744.
(20) Boden, G.; Shulman, G. I. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and *β*-cell dysfunction. *Eur. J. Clin. Invest.* **2002**, *32* (Suppl. 3), 14−23.
(21) Zammit, V. A. Regulation of ketone body metabolism. *Diabetes Rev.* **1994**, *2*, 132−154.
(22) Shinagawa, S.; Kanamaru, T.; Harada, S.; Asai, M.; Okazaki, H. Chemistry and inhibitory activity of long chain fatty acid oxidation of emeriamine and its analogues. *J. Med. Chem.* **1987**, *30*, 1458−1463.
(23) Venerando, R.; Miotto, G.; Kadowaki, M.; Siliprandi, N.; Mortimore, G. E. Multiphasic control of proteolysis by leucine and alanine in the isolated rat hepatocyte. *Am. J. Physiol.* **1994**, *266*, C455−C461.
(24) Zammit, V. A.; Fraser, F.; Padovese, R. Distinct Kinetics of Carnitine Palmitoyltransferase I in Contact Sites and Outer Membranes of Rat Liver Mitochondria. *J. Biol. Chem.* **2001**, *276* (23), 20182−20185.
(25) Jackson, V.; Cameron, J. M.; Fraser, F.; Zammit, V. A.; Price, N. T. Use of Six Chimeric Proteins To Investigate the Role of Intramolecular Interactions in Determining the Kinetics of Carnitine Palmitoyltransferase I Isoforms. *J. Biol. Chem.* **2000**, *275* (26), 19560−19566.

JM020979U