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Letters

Reversible Carnitine Palmitoyltransferase Inhibitors with Broad Chemical Diversity as Potential Antidiabetic Agents

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Abstract: A series of carnitine related compounds of general formula $XCH_2CHZRCH_2Y$ were evaluated as CPT I inhibitors in intact rat liver (L-CPT I) and heart mitochondria (M-CPT I). Derivative **27** (ZR = $-HNSO_2R$, R = C_{12}, X = trimethylammonium, Y = carboxylate, (*R*) form) showed the highest activity (IC_{50} = 0.7 μM) along with a good selectivity (M-CPT I/L-CPTI IC_{50} ratio = 4.86). Diabetic db/db mice treated orally with **27** showed a significant reduction of serum glucose levels.

Introduction. The contribution of hepatic gluconeogenesis to the fasting and post-absorptive hyperglycemia of type II diabetes is well established.[1] This appears to be related to the lack of suppressive effects of both hyperglycemia and hyperinsulinemia on hepatic glucose production. It is also thought that the high level of plasma free fatty acids (FFA) leads to an increase of liver mitochondrial β-oxidation, which is crucial to drive gluconeogenesis at higher rates.[2] The mitochondrial oxidation of long-chain FFA requires the intervention of two membrane-bound carnitine-dependent long-chain acyltransferases, also known as carnitine palmitoyltransferases (CPT).[3] CPT I, the outer mitochondrial membrane enzyme, catalyzes the formation of long-chain acylcarnitines. Two genes encode for what are known as liver (L-CPT I) and muscle (M-CPT I) isoforms of CPT I. CPT II, the inner mitochondrial membrane enzyme, reconverts long-chain acylcarnitines into long-chain acyl coenzyme A esters. At variance of CPT I, CPT II is the product of a single gene. Long-chain acyl-CoAs are then β-oxidized to acetyl-coenzyme A, which activates a key gluconeogenetic enzyme, pyruvate carboxylase.[4] CPT inhibitors, by lowering the level of acetyl-coenzyme A, indirectly reduce liver gluconeogenesis and are hence helpful in the treatment of type II diabetes as hypoglycemic agents.[5,6] Methyl 2-tetradecylglycidate is the first of a class of mitochondrial irreversible CPT I inhibitors containing the oxirane carboxylate moiety.[5] The clinical development of these compounds as hypoglycemic agents was discontinued, possibly because they are equally active in inhibiting both L- and M-CPT I isoforms, and side effects have been observed such as cardiac hypertrophy.[7]

Recent studies have reported on new carnitine derivatives as reversible CPT I inhibitors and good hypoglycemic agents.[8,9] These compounds were designed as transition state mimics, assuming a mechanism similar to that reported for the acyl transfer with the highly homologous enzyme carnitine acetyltransferase. Reversible CPT I inhibition, selective for L-CPT I versus M-CPT I isoform, resulted in an improved approach for pharmacological intervention on fatty acid oxidation, with indirect glycemic control without induction of myocardial hypertrophy.[8]

Following this general strategy, and considering that no crystallographic data are available for CPT I, we

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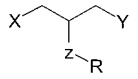
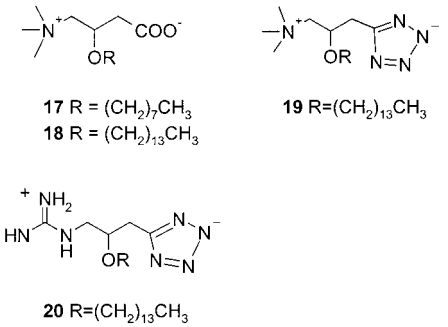


Figure 1. General formula of racemic compounds; X, Y, and Z groups were chosen on the basis of their chemical diversity and synthetic accessibility. X: trimethylammonium, trimethylphosphonium, pyridinium, quinuclidinium, guanidinium. Y: carboxylate, phosphonate, tetrazolate. Z: oxygen, amino, ureido, thioureido, carbamate, carbonate, sulfonamide, sulfamide. R: C_8-C_{10} and C_{14} linear alkyl.



17 R = $(CH_2)_7CH_3$
18 R = $(CH_2)_{13}CH_3$
19 R=$(CH_2)_{13}CH_3$
20 R=$(CH_2)_{13}CH_3$

Figure 2. Structures of compounds **17**−**20**.[14]

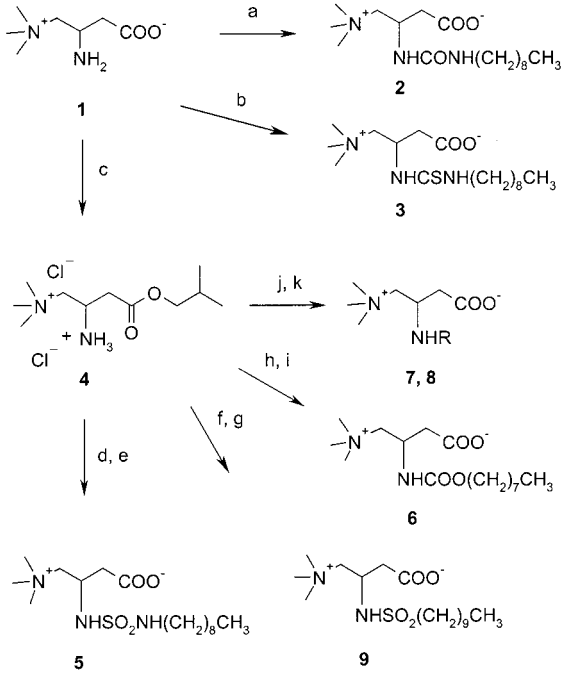
reasoned that carnitine analogues, with medium to long alkyl chains, could occupy the catalytic site of the enzyme, thus slowing down the rate of conversion of long-chain acyl-CoA into long-chain acylcarnitines.[10,11] Thus, we have designed, synthesized, and evaluated the pharmacological properties of a series of racemic carnitine related compounds as potential reversible CPT I inhibitors.

Results and Discussion. Figure 1 shows the general formula of carnitine related compounds, where X, Y, and Z groups were chosen on the basis of their chemical diversity and synthetic accessibility. Only 18 compounds were selected of the possible 270 combinations, also employing the experimental D-optimal design approach,[12] and finally synthesized.[13] The effects of chirality were also investigated for a few selected compounds. The structures of the compounds as well as the synthetic procedures are summarized in Figure 2 and in Schemes 1−4.

As reported in Scheme 1, aminocarnitine derivatives have been synthesized, in the case of compounds **2** and **3**, from racemic aminocarnitine **1**,[14] or from its isobutyl ester **4** for compounds **5**−**9**. The synthesis of the carnitine analogues **11** and **13**, obtained from (*R*,*S*)-4-iodo-3-hydroxybutyrate esters **10**[15] and **12**,[16] and of compounds **15** and **16** starting from racemic carnitine perchlorate benzyl ester **14**,[17] is reported in Scheme 2. The structures of compounds **17**−**20** are reported in Figure 2.[18] Starting from epibromhydrine **21**, derivatives **23**−**25** have been obtained as illustrated in Scheme 3.

IC_{50} (μM) values for L-CPT I and M-CPT I are reported in Table 1. M-CPT I inhibition was determined only for compounds characterized by $IC_{50} < 25$ μM in rat liver mitochondria. It should be noted that the M-CPT I isoform is expressed in both cardiac and skeletal muscle. Aminocarnitine derivatives (X = trimethylammonium, Y = carboxylate) with Z = carbamate (**6**), ureido (**2**), sulfonamide (**9**), and sulfamide (**5**) were shown to be the most active compounds. The IC_{50} values for the liver isoform were better than, or comparable to, that of the reference compound SDZ-CPI 975,[9] with

Scheme 1[a]



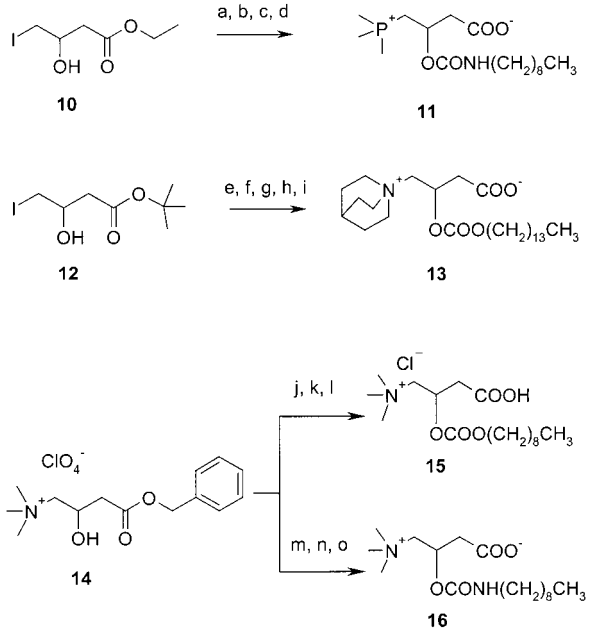
[a] Reagents, conditions, and yields: (a) 2 $CH_3(CH_2)_8NCO$, DMSO, 40 °C, 60 h, 68%; (b) 2 $CH_3(CH_2)_8NCS$, DMSO, 40 °C, 60 h, 53%; (c) HCl(g) in isobutyl alcohol, 130 °C, 18 h, 95%; (d) 3 SO_2Cl_2, 4 NEt_3, CH_2Cl_2, 3 h, then 2 NEt_3, 2 $CH_3(CH_2)_8NH_2$, 18 h; (e) 1 N NaOH, 18 h, 50% (from step d); (f) 2.5 $CH_3(CH_2)_9SO_2Cl$, 5.5 NEt_3,CH_2Cl_2, 72 h; (g) 1 N NaOH, 18 h, 44% (from step f); (h) 1.5 $CH_3(CH_2)_7OCOCl$, 9 NEt_3, CH_2Cl_2, 4.5 h, 50%; (i) IRA 402 resin (OH−), 94%; (j) 1.1 R^1CHO, MeOH, CH_3COOH, Pd/C H_2 (30 psi), 18 h, 47% ($R^1 = CH_3(CH_2)_6$ for **7**, and $R^1 = CH_3(CH_2)_{12}$ for **8**); (k) IRA 402 resin (OH−), 70% (R = $CH_3(CH_2)_7$ for **7** and R = $CH_3(CH_2)_{13}$ for **8**).

selectivity for heart versus liver isoform (M-CPT I/L-CPT I IC_{50} ratio) ranging from 1.4 (compound **9**) to 9.5 (compound **6**). The ureido derivative **2** showed a 4.2 selectivity, while for SDZ-CPI 975 a 3.6 value was found.

When X = trimethylammonium and Y = carboxylate (aminocarnitine and carnitine derivatives), the great influence of the Z group became clear by comparing the inhibitory activity of the two carbamate moieties NH-COO (**6**, IC_{50} = 22,3 μM) and OCONH (**16**, $IC_{50} > 300$ μM), of the two ureido (Z = NHCONH, **2**, IC_{50} = 19.5 μM) and thioureido moieties (Z = NHCSNH, **3**, IC_{50} = 122 μM), and of carbonate too (**15**, $IC_{50} > 300$ μM).

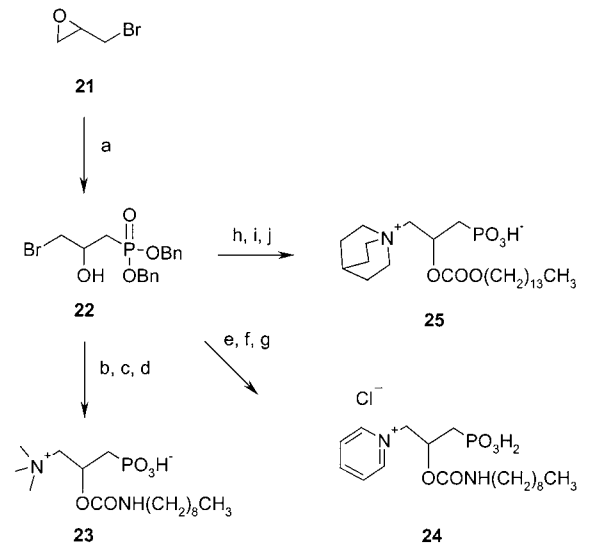
On the grounds of these encouraging results, we decided to investigate the effects of chirality and alkyl chain length on the most active L-CPT I inhibitors, namely sulfonamide **9** and sulfamide **5**. Thus, starting from aminocarnitine of the corresponding configuration[14] (Scheme 4), we have synthesized derivatives **26** and **27**, the *S* and *R* sulfonamide compounds (R = C_{12}), respectively, and **28** and **29**, the *S* and *R* sulfamide compounds, respectively (R = C_{11}). Table 2 shows that the *R* derivatives **27** and **29** are more effective L-CPT I inhibitors than the *S* ones (compounds **26** and **28**), even by comparison with their original racemic derivatives. In addition, an improvement of selectivity of *R* forms toward the L-CPT I isoform was also observed. The M-CPT I/L-CPT I IC_{50} ratios were 4.8 (**27**) and 7.2 (**29**). Even if these results were not totally unexpected, they confirm that *R* derivatives are more active than *S* ones with all the examined functional groups, and that the

Scheme 2[a]



[a] Reagents, conditions, and yields: (a) 1.2 $(Me)_3P$, THF, 120 h, 71%; (b) 2 $CH_3(CH_2)_8NCO$, DMF, 110 °C, 7 d, 34%; (c) 1 N HCl, 70 °C, 3 h; (d) Amberlyst A-21, 49% (from step c); (e) 1 quinuclidine, CH_3CN, 60 °C, 20 h, 50%; (f) 1.5 $CH_3(CH_2)_{13}OCOCl$, 1.5 DMAP, CH_2Cl_2, 20 h; (g) Amberlyst A-21 (HCl form), 58% (from step f); (h) trifluoroacetic acid, 1 h; (i) IRA 402 (Cl^-), SiO_2 gel flash chromatography, 55% (from step h); (j) 1 DMAP, 1 $CH_3(CH_2)_8OCOCl$, DMF, 72 h; (k) A-21 resin (HCl form), 32% (from step j); (l) Pd/C 10%, H_2 (47 psi), MeOH, 2 h, 68%; (m) 2 $C_9H_{19}NCO$, toluene, reflux, 120 h, then 0.5 $C_9H_{19}NCO$, toluene, reflux, 120 h, 38%; (n) Pd/C 10%, H_2 (47 psi), 4 h, MeOH; (o) Amberlyst A-21 resin, 88% (from step n).

Scheme 3[a]



[a] Reagents, conditions, and yields: (a) 1 $HPO(OBn)_2$, 1 BuLi, 1 BF_3Et_2O, THF, −70 °C, 3 h, 60%; (b) 2 $CH_3(CH_2)_8NCO$, 1.25 BF_3Et_2O, CH_2Cl_2, 30′, 85%; (c) trimethylamine (gas), TBAI cat. DMF, 50 °C, 24 h, 25%; (d) Pd/C 10%, H_2 (60 psi), MeOH, 18 h, 99%; (e) 2 $CH_3(CH_2)_8NCO$, 1.25 BF_3Et_2O, CH_2Cl_2, 30′, 85%; (f) TBAI cat., pyridine, 50 °C, 24 h, 20%; (g) 2 $(CH_3)_3SiI$, CH_2Cl_2, 30′, then H_2O, IRA 402 (Cl^-), 80%; (h) 1 $CH_3(CH_2)_{13}OCOCl$, 1 DMPA, 2 TEA, $CHCl_3$, 24 h, 75%; (i) 2 quinuclidine, TBAI cat., DMF, 50 °C, 24 h, 15%; (j) Pd/C 10%, H_2 (60 psi), MeOH, 18 h, 99%.

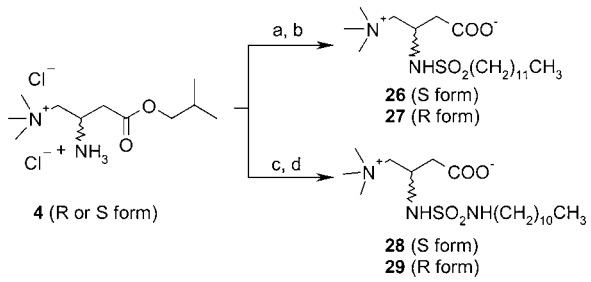
longer alkyl chains seem to induce stronger inhibitory capability. Furthermore, a kinetic study revealed that

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

compd	chain length (carbon atoms)	IC_{50}[b] liver (μM)	IC_{50}[b] heart (μM)
2	9	19.5	83.2
3	9	122	
5	9	2.3	3.8
6	8	22.3	211
7	14	230	
8	8	>300	
9	10	1.6	2.3
11	9	>300	
13	14	>300	
15	9	>300	
16	9	>300	
17	8	>300	
18	14	47	
19	14	>300	
20	14	not determined (insoluble)	
23	9	>300	
24	9	>300	
25	14	>300	
SDZ-CPI975[7,8]	14	17.4	62

[a] The activity of CPT 1 was measured radiochemically using [^{14}C]palmitoylCoA and carnitine itself by evaluating the incorporation of [^{14}C]palmitoyl residue on carnitine in intact fresh liver and heart mitochondria from Sprague−Dawley male rats.[21,22] [b] The IC_{50} values (μM) were calculated from the inhibition curve. Data are means of two determinations in triplicate. The variation between experiments was less than 8%.

Scheme 4[a]



[a] Reagents, conditions, and yields: (a) 2.5 $CH_3(CH_2)_{11}SO_2Cl$, 5.5 NEt_3, CH_2Cl_2, 72 h; (b) 1 N NaOH, 18 h, 40% (from step a); (c) 3 SO_2Cl_2, 4 NEt_3, CH_2Cl_2, 3 h, then 2 NEt_3, 2 $CH_3(CH_2)_{10}NH_2$,18 h; (d) 1 N NaOH, 18 h, 35% (from step c).

Table 2. Effect of Sulfonamidic and Sulfamidic Aminocarnitine Derivatives as Inhibitors of Mitochondrial CPT I Activity

compd	chirality	chain length (carbon atoms)	IC_{50}[b] liver (μM)	IC_{50}[b] heart (μM)
26	*S*	12	6	>10
27	*R*	12	0.7	3.4
28	*S*	11	6	>10
29	*R*	11	0.8	5.8

[a] The IC_{50} values (μM) were calculated from the inhibition curve. Data are means of two determinations in triplicate. The variation between experiments was less than 8%.

27 is a reversible, mixed inhibitor of L-CPTI with respect to palmitoyl-CoA (apparent $K_i = 0.25 \pm 0.01$ μM).

An initial in vivo test designed to evaluate the efficiency of a CPT I inhibitor can be conveniently carried out by determining plasma β-hydroxybutyrate (BHB) levels in fasted animals. Since BHB is a major product of liver fatty acid oxidation during fasting,[19] a liver specific CPT I inhibitor would significantly reduce circulating BHB levels. According to the in vitro findings on L-CPT I inhibition, when compound **27** was orally

administered to 24 h fasted normal rats, a reduction of plasma BHB at 6 h postdose with an ED_{50} of 20 mg/kg occurred. This finding prompted us to test the antidiabetic activity of **27** on db/db mice, a model of type II diabetes.[20] The oral administration of **27** for 30 days at a dose of 100 mg/kg daily caused a significant reduction of serum glucose levels with respect to untreated db/db mice (528 ± 45.8 vs 734 ± 47.6 mg/dL; mean $\pm$ SE; $p < 0.01$). In addition, such a treatment did not affect either body weight or circulating concentrations of insulin, triglyceride, urea, alanine aminotransferase, and cholesterol compared to controls. Moreover, cardiac and liver sizes (heart and liver/body weight ratios) were similar in both experimental groups.

Further investigation will be devoted to explore the effects of chain length and chirality, in terms of activity and selectivity, in the case of different functional groups (for example, ureidic and carbamic ones) and the in vivo effects of selected compounds as antichetotic and potential antidiabetic agents in several animal models.

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Supporting Information Available: Analytical data are available free of charge via the Internet at http://pubs.acs.org.

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