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STEREOISOMERIC ACYLAMIDOMORPHOLINIUM CARNITINE ANALOGUES: SELECTIVE INHIBITORS OF CARNITINE PALMITOYLTRANSFERASE I AND II

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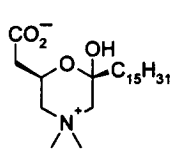
Abstract: Acylamidomorpholinium carnitine analogues, 6-(tetradecanamidomethyl- and -hexadecanamidomethyl)-4,4-dimethylmorpholin-4-ium-2-acetate, **1**, synthesized as complete sets of stereoisomers, were assayed as inhibitors for isozymes of carnitine palmitoyltransferase (CPT). Microsomal CPT isozymes showed modest discrimination among the stereoisomers; while rat-liver mitochondrial CPT-I and CPT-II showed distinct differences. The tetradecanamidomethyl analogue of (2*R*,6*S*)-**1** activated CPT-I but inhibited CPT-II.

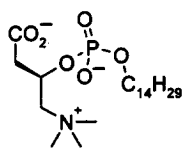
Carnitine palmitoyltransferases (CPTs) catalyze transfer of fatty acyl groups to and from coenzyme A. Mitochondrial CPT-I and CPT-II catalyze key steps in supplying fatty acyl groups to the fatty acid oxidation (FAO) cascade in the mitochondrial matrix. Controlling FAO can regulate blood-glucose levels and ameliorate some symptoms of Type II (noninsulin-dependent) diabetes milletus (NIDDM), a condition that accounts for over 90% of the cases of diabetes.[1] Inhibitors of strategic enzymes in the lipid metabolic pathway can decrease FAO and, therefore, serve as adjuvant therapeutic agents to help manage NIDDM.[2] Isozyme-selective inhibitors offer the potential of minimizing undesirable side effects.[2]

CPT isozymes also exist in peroxisomes[3] and microsomes.[4] Isozymes from these organelles resemble the mitochondrial isoforms in that one CPT is malonyl-CoA sensitive and the other is not. In peroxisomes and microsomes, the functions of CPT isozymes remain a mystery. Availability of isozyme-selective inhibitors also would help reveal the function of the various CPT isozymes in different organelles.

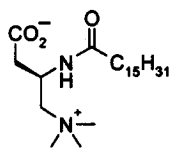
Rationale for Design of CPT Inhibitors. One approach to formulating an effective NIDDM drug is to design selective inhibitors of liver mitochondrial CPTs. Anderson[2] points out that the liver CPT-I isoform is a better target than CPT-II. CPT-II is the same enzyme in all tissues; however, liver CPT-I differs from muscle CPT-I. The cyclohexyl carnitine analogue (CC) binds selectively to the active site of CPT-I.[5] Reversible inhibitors of CPT have a long-chain acyl group linked to a carnitine moiety (e.g., HPC,[6] SDZ-CP1-975[7]) or aminocarnitine moiety (e.g., N-PAC[8] and SDZ-269-456[2]). Furthermore, N-PAC



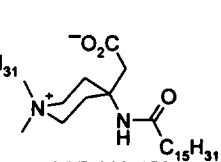
HPC



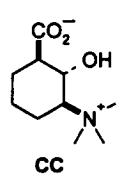
SDZ-CPI-975



N-PAC



SDZ-269-456



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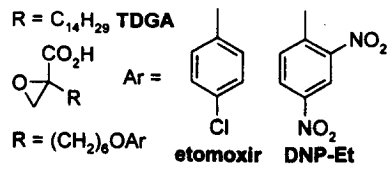
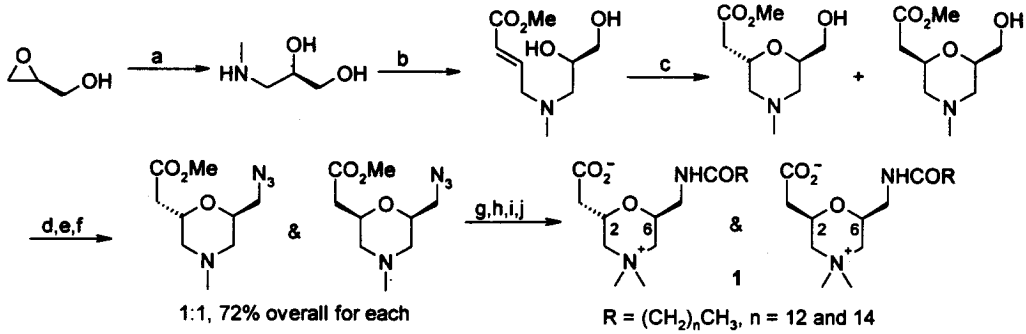
forms when CPT reacts with palmitoyl-CoA and (*R*)-aminocarnitine, which is the microbial metabolite emeriamine.[9] Irreversible inhibitors of CPT use a glycidic-acid design, an epoxide with an alkyl chain and a carboxyl group (e.g., TDGA,[10] etomoxir,[11] and DNP-Et[12]). DNP-Et selectively inhibits liver CPT-I.[13]



Following our syntheses[14] of stereoisomeric inhibitors of carnitine acetyltransferase, we have synthesized **1**, an 'expanded' aminocarnitine analogue, to screen isozymes of CPT for selective inhibition by different stereoisomers and different acyl groups. CPT isozymes might selectively bind different stereoisomers of **1**. Although isozymes catalyze the same reaction, the stereochemical arrangements for recognition of the carnitine and acyl moieties may vary among isozymic active sites.

Synthesis.[15] Scheme 1 describes the synthesis of the stereoisomers of **1**, which were fully characterized.

Scheme 1.



a. xs $MeNH_2$, EtOH; b. $BrCH_2CH{=}CHCO_2Me$, THF, K_2CO_3; c. THF/MeOH (1:1); d. MsCl, NEt_3, DMAP, CH_2Cl_2; e. NaN_3, DMF, 90 °C; f. Chromatography on silica gel; g. 10% Pd/C, H_2, MeOH; h. RCOCl, NEt_3, DMAP, PhMe, Δ; i. MeI, CH_3NO_2; j. NaOH, RP-8 chromatography.

Ring-opening of (*R*)- and (*S*)-glycidol with an excess of methylamine gives an aminodiol in 98% yield, which equals a previous two-step, one-pot procedure with *N*-methylbenzylamine and racemic glycidol.[16] Construction of the morpholine alcohols follows our previous procedure:[14] an intramolecular conjugate addition (step c) preceded by nucleophilic substitution (step b). Among the various synthetic intermediates, separation of the diastereomeric azidomethylmorpholines occurs most readily. Conversion of an azidomethylmorpholine into the corresponding stereoisomer of **1** in four steps occurs in 80% yield for either chain length of acyl chloride.

CPT Assays. We assayed CPT as the conversion of ^{14}C-palmitoyl-CoA into ^{14}C-palmitoylcarnitine. Reactions were initiated by the addition of CPT, incubated at 30 °C for 5 min, and stopped by addition of MeOH. As described previously,[17] ^{14}C-palmitoylcarnitine was separated. For Table 1, rat-liver mitochondrial membranes were prepared.[18] For Table 2, microsomal soluble and pellet fractions were isolated.[4]

Results. Stereoisomers of **1** inhibit CPT-I more strongly than CPT-II. (Table 1) All stereoisomers of both palmitoyl and myristoyl analogues **1** strongly inhibit rat-liver mitochondrial CPT-I. This concentration (500 μM) of inhibitor may obscure any potential selectivity, either for stereoisomer or for chain length, for CPT-I. In

contrast, the stereoisomers selectivity inhibit rat-liver mitochondrial CPT-II (Table 1). Stereoisomer (2*S*,6*R*)-**1** inhibits the best; the palmitoyl analogue is slightly better than the myristoyl one. The palmitoyl analogue of (2*R*,6*S*)-**1** barely inhibits CPT-II, while the myristoyl analogue activates it!

Table 1. Inhibition[a] of Rat-liver Mitochondrial CPTs by Stereoisomers of **1**.

	CPT II		CPT I	
Stereoisomer	n = 12	n = 14	n = 12	n = 14
Methanol (vehicle)	+14.1 ± 3.7%		+ 40 ± 10.1%	
(2*R*, 6*S*)-**1**	+41 ± 4.6%	-15 ± 7.0%	-91± 0.9%	-88 ± 3.5%
(2*S*, 6*S*)-**1**	-19 ± 6.9 %	-72 ± 6.2%	-90 ± 4.9 %	-96 ± 2.0%
(2*S*, 6*R*)-**1**	-64 ± 7.6 %	-85 ± 3.5 %	-93 ± 4.1 %	-90 ± 7.3%
(2*R*, 6*R*)-**1**	-40 ± 16.6%	-62 ± 3.8%	-94 ± 4.4%	-93 ± 1.9%

[a] Incubation solution (100 μL) contained 220 mM sucrose, 40 mM KCl, 10 mM Tris-HCl, pH 7.4, 2 mL/mg bovine serum albumin, 1 mM carnitine, 50 μM ^{14}C-palmitoyl-CoA, 500 μM of **1**. Mean ± SEM of 3 to 5 data points are shown. Percentage effects are based on comparisons of simultaneously carried out vehicle (methanol) matched controls.

The stereoisomers of **1** inhibit both soluble and membrane-bound microsomal CPT. (Table 2) Among the palmitoyl analogues, (2*S*,6*S*)-**1** inhibits these two CPT isozymes most strongly. Among the myristoyl analogues, (2*R*,6*S*)-**1** most strongly inhibits both. The membrane-bound enzyme shows modest selectivity among the myristoyl analogues. The strong activation by methanol may obscure how well these analogues inhibit the microsomal CPTs.

Table 2. Inhibition[a] of Soluble and Membrane-bound Microsomal CPT by Stereoisomers of **1**.

Compound	soluble		membrane bound	
	n = 12	n = 14	n = 12	n = 14
MeOH(vehicle)	+36%	+36%	+68%	+68%
(2*R*, 6*S*)-**1**	-69%	-56%	-74%	-54%
(2*S*, 6*S*)-**1**	-50%	-77%	-31%	-81%
(2*S*, 6*R*)-**1**	-49%	-60%	-36%	-56%
(2*R*, 6*R*)-**1**	-56%	-58%	-33%	-72%

[a] Incubation solution (100 μL) contained 220 mM sucrose, 40 mM KCl, 10 mM Tris-HCl, pH 7.4, 2 mL/mg bovine serum albumin, 1 mM carnitine, 50 μM ^{14}C-palmitoyl-CoA, 500 μM of **1**; runs in triplicate

Discussion. Tables 1 and 2 present data on a rapid assay of inhibition of CPT isozymes. Data for inhibition of microsomal CPTs (Table 2) show modest discrimination among the stereoisomers; data for inhibition of rat-liver mitochondrial CPT-I and CPT-II (Table 1) show distinct differences among the stereoisomers.

These morpholinium analogues inhibit CPT-I more effectively than CPT-II. This difference in inhibition contrasts that of HPC; the latter inhibits CPT-II in the sub-micromolar range[19] and CPT-I in the micromolar range.[6] HPC has the same relative configuration as (2*R*,6*S*)-**1**, which is the least effective inhibitor for CPT-II among the palmitoyl and myristoyl analogues. Strikingly, (2*R*,6*S*)-**1** (n = 12) activates CPT-II. Part of the activation arises from the solvent (methanol) for the inhibitors. Compound (2*R*,6*S*)-**1** (n = 12) emerges as a potentially useful compound for the selective inhibition of CPT-I.

Drug Development. In order for these analogues to treat NIDDM, they must overcome the challenges described in Anderson's trenchant review.[2] Biological studies by Anderson and his colleagues[2] of liver-selective CPT-I reversible inhibitors reveal "hepatic mitochondrial aberrations". The irreversible inhibitors, glycidic acids, are no longer in development, likely because of myocardial hypertrophy.[2] Anderson rightfully concludes, "major issues would need to be more critically examined before committing to full development."

Developing isozyme-selective inhibitors of CPT remains a viable goal for the following reasons: (1) CPTs have functions beyond the liver, and (2) acylcarnitines can modulate the activity of other enzymes. For

example, two isozymes of CPT-I have roles in sperm maturation;[20] a selective CPT inhibitor might serve as a male contraceptive agent. Palmitoylcarnitine inhibits protein kinase C in neuroblastoma NB-2a cells;[21] a palmitoylcarnitine analogue might inhibit tumor-cell proliferation. Palmitoylcarnitine, a lysophospholipase-transacylase inhibitor,[22] interferes with Candida adherence to lysophospholipids and the HEp-2 cell line;[23] a palmitoylcarnitine analogue might serve as an antimicrobial agent.[24] Consequently, continued development of acylcarnitine analogues promises to offer benefits beyond controlling NIDDM.

Constructing a scaffold on which to build a diverse set of stereoisomers combines both classic and modern approaches to drug design. The classic approach is to construct a conformationally constrained substrate (product) analogue; the modern approach is to synthesize a complete set of stereoisomers. A combined approach can identify any topographical changes that occur in binding sites of various isozymes. Most CPT inhibitors inhibit CPT-I profoundly; the challenge now is to design selective inhibitors for each CPT isozyme.

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References

1. *Diabetes In America*, 2nd edit.; National Diabetes Data Group, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases NIH Publication No. 95-1468, Bethesda, MD, 1995.
2. Anderson, R. C. *Curr. Pharm. Design* **1998**, *4*, 1.
3. Derrick, J. P.; Ramsay, R. R. *Biochem. J.* **1989**, *262*, 801.
4. Murthy, M. S. R.; Pande, S. V. *J. Biol. Chem.* **1994**, *269*, 18283.
5. Hutchinson, T. L.; Saeed, A.; Wolkowicz, P. E.; McMillin, J. B.; Brouillette, W. J. *Bioorg. Med. Chem.* **1999**, *7*, 1505.
6. Gandour, R. D.; Leung, O-t.; Greway, A. T.; Ramsay, R. R.; Níc a' Bháird, N.; Fronczek, F. R.; Bellard, B. M.; Kumaravel, G. *J. Med. Chem.* **1993**, *36*, 237.
7. 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. *J. Med. Chem.* **1995**, *38*, 3448.
8. Jenkins, D. L.; Griffith, O. W. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 290.
9. Shinagawa, S.; Kanamaru, T.; Harada, S.; Asai, M.; Okazaki, H. *J. Med. Chem.* **1987**, *30*, 1458.
10. Tutwiler, G. F.; Dellevigne, P. *J. Biol. Chem.* **1979**, *254*, 2935.
11. Lopaschuk, G. D.; Wall, S. R.; Olley, P. M.; Davies, N. J. *Circ. Res.* **1988**, *63*, 1036.
12. Hutchison, T. L.; Brouillette, W. J. *Bioorg. Med. Chem.* **1998**, *6*, 2133.
13. Weis, B. C.; Cowan, A. T.; Brown, N.; Foster, D. W.; McGarry, J. D. *J. Biol. Chem.* **1994**, *269*, 26443.
14. Sun, G.; Savle, P. S.; Gandour, R. D.; Níc a' Bhaird, N.; Ramsay, R. R. *J. Org. Chem.* **1995**, *60*, 6688.
15. Experimental details are available from the authors upon request.
16. Thompson, A. M.; Murray, D. K.; Elliott, W. L.; Fry, D. W.; Nelson, J. A.; Hollis Showalter, H. D.; Roberts, B. J.; Vincent, P. W.; Denny, W. A. *J. Med. Chem.* **1997**, *40*, 3915.
17. Pande, S. V.; Murthy, M. S. R.; Noel, H. *Biochem. Biophys. Acta* **1986**, *877*, 223.
18. Murthy, M. S. R.; Pande, S. V. *Biochem. J.* **1987**, *248*, 727.
19. Níc a' Bháird, N.; Kumaravel, G.; Gandour, R. D.; Krueger, M. J.; Ramsay, R. R. *Biochem. J.* **1993**, *294*, 645.
20. Adams, S. H., Esser, V.; Brown, N. F.; Ing, N. H.; Johnson, L.; Foster, D. W.; McGarry, J. D. *Biol. Reprod.* **1998**, *59*, 1399.
21. Nalecz, K. A.; Mroczkowska, J. E.; Berent, U.; Nalecz, M. J. *Acta Neurobiol. Exp. (Warsz.)* **1997**, *57*, 263.
22. Gross, R. W.; Sobel, B. E. *J. Biol. Chem.* **1983**, *258*, 5221.
23. Prakobphol, A.; Leffler, H.; Hoover, C. I.; Fisher, S. J. *FEMS Microbiol. Lett.* **1997**, *151*, 89.
24. Savle, P. S.; Doncel, G. F.; Bryant, S. D.; Hubieki, M. P.; Robinette, R. G.; Gandour, R. D. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2545.