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New fatty acid oxidation inhibitors with increased potency lacking adverse metabolic and electrophysiological properties

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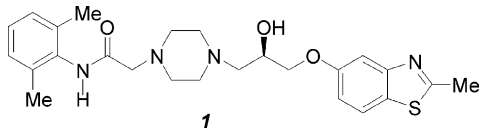
Abstract—New inhibitors of palmitoylCoA oxidation were synthesized based on a structurally novel lead, CVT-3501 (**1**). Investigation of structure–activity relationships was conducted with respect to potency of inhibition of cardiac mitochondrial palmitoylCoA oxidation and metabolic stability. Potent and metabolically stable analogues **33**, **42**, and **43** were evaluated in vitro for cytochrome P450 inhibition and potentially adverse electrophysiological effects. Compound **33** was also found to have favorable pharmacokinetic properties in rat.

Fatty acids and glucose are the two major sources of energy utilized by cardiac muscle. Compounds that decrease fatty acid oxidation and subsequently increase the rate of glucose oxidation are of potential therapeutic importance for the treatment of ischemic heart disease.[1–3]

(*R*)-CVT-3501 (**1**, Fig. 1) was found to be an inhibitor of oxidation of 1-[^{14}C]-palmitoylCoA, a substrate for long-chain fatty acid oxidation (palmitoylCoA oxidation inhibitor) in cardiac mitochondrial preparations isolated from rat heart (IC_{50}: 2.5 μM).[4] It was reasonably metabolically stable with 50% remaining after 30 min incubation with human isolated liver S9 preparations.

We conducted a study to investigate the structure–activity relationships in the aryl amide portion of our lead in order to optimize the potency with respect to palmitoylCoA oxidation inhibition and improve metabolic stability.



1

Figure 1. Structure of (*R*)-CVT-3501.

Synthesis of analogues of **1** is outlined in Scheme 1. Commercially available 5-hydroxybenzothiazole was reacted with (*S*)-epichlorohydrin under standard conditions[5] to provide the desired (*R*)-epoxide **2** in 60% yield, >99% ee.[6] The epoxide was reacted with Boc-piperazine and the Boc-group was removed. The resulting piperazine **3** underwent coupling with corresponding chloroacetamides in a library format under solution-phase[7,8] or resin-mediated conditions.[9]

Initially all the compounds were screened at 30 μM for their activities in palmitoylCoA oxidation inhibition assay. If the compounds inhibited palmitoylCoA oxidation >50% their IC_{50} values were determined, otherwise % inhibition is reported. Liver S9 stability has been determined for all compounds that had IC_{50} values under 30 μM. Results are presented in Table 1.

The change of substitution in the aromatic ring of the amide portion (**4**–**12**), insertion of methylene (**13** and **14**) or saturation (**15** and **16**) did not increase potency. Introduction of a methyl group in the benzyl position (**20**–**24**) showed clear preference for (*S*)-configuration

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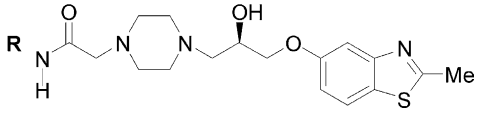
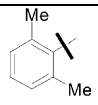
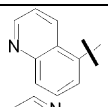
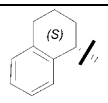
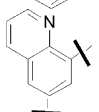
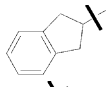
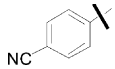
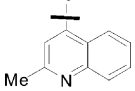
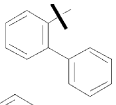
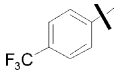
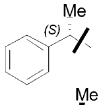
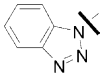
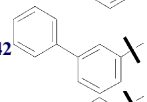
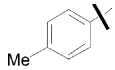
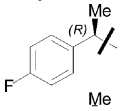
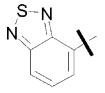
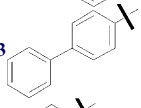
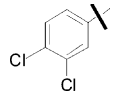
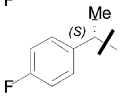
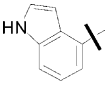
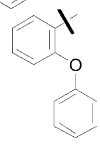
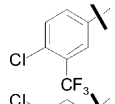
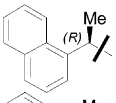
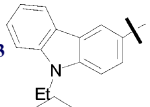
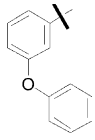
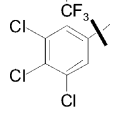
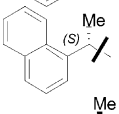
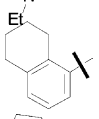
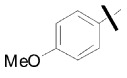
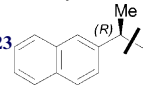
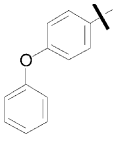
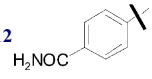
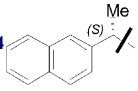
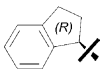
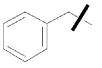
and led to a potent compound (**24**, IC_{50}: 150 nM). The stability of **24**, however was very low (14%). Homobenzylic substitution was not favorable (**25**, IC_{50}: 17 μM). Naphthalene analogue **26** was very potent (IC_{50}: 105 nM) and reasonably stable (50%), but its further evaluation was not possible because of low aqueous solubility.

A set of naphthalene analogues (**27**–**40**) was synthesized in an attempt to improve aqueous solubility while maintaining high potency to inhibit palmitoylCoA oxidation and liver S9 stability. Introduction of a basic nitrogen atom (**27**–**31**) reduced the potency, but a non-basic nitrogen was tolerated (indole **32** and carbazole **33**). Compound **33** also had favorable stability (62%). Partial reduction of the aromatic system maintained potency in some cases but reduced liver S9 stability (**34**–**40**). In all cases, analogues with (*S*)-configuration in the aryl amide portion had higher inhibitory activity than (*R*).

Table 1. PalmCoA inhibition activity and liver S9 stability of mono- and bicyclic amides



	Substituent	Activity[a] (stability)[b]		Substituent	Activity[a] (stability)[b]		Substituent	Activity[a] (stability)[b]		Substituent	Activity[a] (stability)[b]
1		2.5 μM (50%)	**15**		8.6 μM (37%)	**27**		4.7 μM (2%)	**39**		77 nM (2%)
4		10 μM (47%)	**16**		5.1 μM (29%)	**28**		4.7 μM (12%)	**40**		30 μM (2%)
5		42% @ 30 μM (N/D)[c]	**17**		23% @ 30 μM (N/D)[c]	**29**		40% @ 30 μM (N/D)[c]	**41**		39% @ 30 μM (N/D)[c]
6		7.6 μM (28%)	**18**		4.4 μM (23%)	**30**		16% @ 30 μM (N/D)[c]	**42**		180 nM (58%)
7		1.7 μM (51%)	**19**		40% @ 30 μM (N/D)[c]	**31**		5.7 μM (11%)	**43**		460 nM (63%)
8		6.0 μM (29%)	**20**		710 nM (10%)	**32**		860 nM (35%)	**44**		34% @ 30 μM (N/D)[c]
9		4.0 μM (25%)	**21**		24% @ 30 μM (N/D)[c]	**33**		850 nM (62%)	**45**		360 nM (15%)
10		22 μM (34%)	**22**		8.5 μM (10%)	**34**		610 nM (2%)			
11		35 μM (41%)	**23**		8.2 μM (45%)	**35**		190 nM (15%)	**46**		330 nM (18%)
12		35% @ 100 μM (N/D)[c]	**24**		150 nM (14%)	**36**		50% @ 30 μM (N/D)[c]			
13		3.9 μM	**25**		17 μM (27%)	**37**		2.2 μM (1%)	**47**		2.0 μM (64%)
14		2.0 μM (16%)	**26**		105 nM (50%)	**38**		27 μM (N/D)[c]			

[a] Activity is expressed as either IC_{50} or % inhibition at listed concentration as obtained from the rat heart mitochondrial PalmCoA assay. Values are presented as average of two or three measurements.

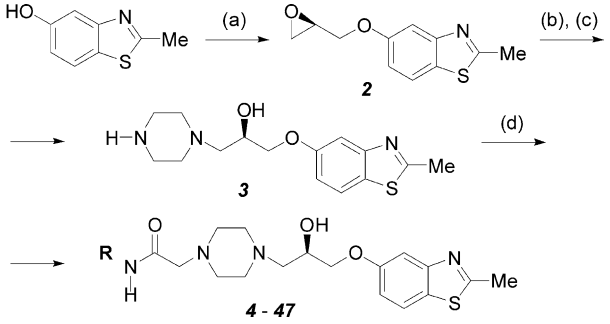
[b] Stability is defined as % remaining after 30 min incubation with human liver S9 ($n = 2$, 2 mg/mL protein, NADH added).

[c] Not determined.

Table 2. Summary of metabolic and electrophysiological properties of **33**, **42**, and **43** compared to the initial lead **1**

Compd	Cytochrome P450 inhibition assay (IC_{50}, μM)			Electrophysiological effects at 3 μM[a]		
	CYP1A2	CYP3A4	CYP2C9	ΔS–H[b]	ΔH–V[b]	ΔCPP[b]
1	>100	23	>100	3.0	4.0	−8.0
33	>100	39	>100	0	0	0.7
33				1.0[c]	0[c]	−0.4[c]
42	6.0	37	24	3.0	0	−4.3
43	31	7.9	30	5.0	0.5	−6.6

[a] In guinea pig isolated hearts, average of $n=2$.
[b] Change in S–H and H–V intervals was measured in ms, change in coronary perfusion pressure was measured in mm Hg.
[c] Measured at 10 μM.



Scheme 1. Reagents and conditions: (a) K_2CO_3, acetone, (*S*)-epichlorohydrine, reflux, 24 h, 60%; (b) Boc-piperazine, CH_2Cl_2, reflux, 24 h, 96%; (c) 50% TFA in CH_2Cl_2, rt, 24 h, 100%; (d) conditions A (solution-phase): R-$NHCOCH_2Cl$, $(i\text{-}Pr)_2NEt$, DMF, 70 °C, 24 h or conditions B (resin-mediated): R-$NHCOCH_2Cl$, PS-DIEA, dichloroethane/DMF, 70 °C, 24 h, then PS-NCO and PS-trisamine, 70 °C, 24 h.

A set of analogues (**41**–**47**) was synthesized with a focus on bulky biphenyl and phenoxyphenyl amides that mimic the tricyclic carbazole system. While *ortho*-substitution led to inactive analogues (**41** and **44**), *meta*- and *para*- phenyl- and phenoxyphenyl-analogues (**42**, **43**, **45**, and **46**) all had potencies of less than 500 nM. Liver S9 stabilities were higher in biphenyl analogues **42** (58%) and **43** (63%) and lower in the phenoxyphenyl analogues **45** (15%) and **46** (18%) which could be alleviated by introduction of 4-chloro substituent (**47**, 64%). This however resulted in a 6-fold decrease in potency.

The best analogues from our study (**33**, **42**, and **43**) were screened for potential effects in isolated guinea pig heart and compared with parent analogue **1** (Table 2). The electrophysiological (EP) parameters measured were AV nodal conduction times including the S–H interval (stimulus to His-bundle conduction time) and H–V interval (His-bundle to ventricular conduction time) measured from His-bundle electrogram. Changes in coronary perfusion pressure (CPP), were also monitored. Prolongation of the S–H interval has been interpreted to suggest inhibition of calcium dependent currents whereas prolongation of the H–V interval has been interpreted to suggest inhibition of sodium dependent currents. Hence, the effect of compounds on these parameters may provide indirect information about its potential effects on membrane ion currents.[10,11] In that respect, properties of all three compounds **33**, **42**, and **43** presented a substantial improvement over **1** when measured at 3 or even 10 μM (**33**) concentrations. The effects of **33**, **42**, and **43** on coronary vasodialation are expected to be minimal based on small changes in ΔCPP observed at concentrations greater than IC_{50} for their respective palmCoA activity.

Table 3. Comparison of pharmacokinetic properties of **1** and **33** in rats

	1		**33**	
	PO[a]	IV[b]	PO[a]	IV[b]
C_{max} (ng/mL)	26.1		26.3	
AUC (0–24 h), (ng h/mL)	61.1	313	230	1315
$t_{1/2}$ (h)	1.2	1.4	2.6	3.0
t_{max} (h)	0.83		4–6	
Cl_p (mL/min/kg)		54.5		13.5
Vd_{ss} (L/kg)		6.2		3.4
F (%)	19.5		17.5	

[a] Dosed at 2 mg/kg, $n=3$.
[b] Dosed at 1 mg/kg, $n=3$.

Analogues **33**, **42**, and **43** were also screened for human cytochrome P450 inhibition and compared with parent analogue **1**.[12–15] Similarly to **1**, compound **33** showed no or only mild cytochrome P450 inhibition, while compounds **42** and **43** were moderately potent inhibitors of CYP1A2 and CYP3A4, respectively.

Comparison of in vivo pharmacokinetic properties of **1** and **33** is shown in Table 3. While having oral bioavailability similar to **1**, **33** demonstrated slower clearance and longer elimination half-life than compound **1** (Table 3) in rats.

In conclusion, we have identified a new fatty acid oxidation inhibitor **33** with increased potency, desirable metabolic stability, low cytochrome P450 inhibition potential, and lack of adverse electrophysiological effects. In addition, **33** had favorable in vivo pharmacokinetic properties. The ability of **33** to alter cardiac energy substrate oxidation has yet to be determined.

Acknowledgements

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References and notes

1. Schofield, R. S.; Hill, J. A. *Am. J. Cardiovasc. Drugs* **2001**, *1*, 23.
2. McCormack, J. G.; Stanley, W. C.; Wolff, A. A. *Gen. Pharm.* **1998**, *30*, 639.
3. McCormack, J. G.; Barr, R. L.; Wolff, A. A.; Lopaschuk, G. D. *Circulation* **1996**, *93*, 135.
4. Nedergaard, J.; Cannon, B. *Methods Enzymol.* **1979**, *55*, 3.
5. Shiratsuchi, M.; Kawamura, K.; Akashi, T.; Ishihama, H.; Nakamura, M.; Takenaka, F. *Chem. Pharm. Bull.* **1987**, *35*, 3691.
6. Enantiomeric excess has been determined by chiral HPLC (Chiralcel OD column, 40 °C, 89.1% hexanes/9.9% EtOH/1% Et_2NH).
7. Oshiro, Y.; Sakurai, Y.; Tanaka, T.; Kikuchi, T.; Hirose, T.; Tottori, K. *J. Med. Chem.* **1991**, *34*, 2014.
8. Solution-phase coupling procedure (conditions A): chloroacetamide (0.14 mmol) and monosubstituted piperazine (solution in DMF, 0.17 mmol, 0.6 mL) were placed into a 4-mL vial and diluted with 1.8 mL DMF. Hunig's base (0.28 mmol) was added via syringe and the vial shaken at 70 °C overnight. Products were isolated in 5–70% yields with purities exceeding 95% (as determined by analytical HPLC) in most cases. Structures of all final products were supported by ESI MS and ^{1}H NMR.
9. Resin-mediated coupling procedure (conditions B): chloroacetamide (0.14 mmol) and monosubstituted piperazine (solution in DMF, 0.17 mmol, 0.6 mL) were placed into a 4-mL vial and diluted with 1.8 mL dichloroethane. PS-DIEA resin (200 mg) was added, and the vial shaken at 70 °C overnight. After cooling to room temperature, 200 mg each of PS-Isocyanate resin and PS-Trisamine resin were added and shaken at 70 °C overnight. After cooling to room temperature, contents of the vial were transferred into a frit-fitted syringe, filtered, resins washed with additional dichloroethane repeatedly. After concentration on Speedvac® the crude mixture was purified with semi-preparative reverse-phase HPLC (ACN/water). Products were isolated in 5–70% yields with purities exceeding 95% (as determined by analytical HPLC) in most cases. Structures of all final products were supported by ESI MS and ^{1}H NMR. All resin-bound reagents were obtained from Argonaut Technologies.
10. Lazzara R.; Scherlag G. J.; Belardinelli L. 2002. Atrioventricular Conduction. In *Foundations of Cardiac Arrhythmias: Basic Concepts and Clinical Approaches*; Spooner, P. M, Rosen, M. R., Eds.; Marcel Dekker; New York, Basel; p 265.
11. Compounds were formulated as x·HCl salts by treatment with excess HCl to assist solubility and administered with 5% DMSO and 0.9 M NaCl.
12. Tassaneeyakul, W.; Birkett, D.; Veronese, M.; McManus, M.; Tukey, R.; Quattrochi, L.; Gelboin, H.; Miners, J. *J. Pharmacol. Exp. Ther.* **1993**, *265*, 401.
13. Scmitz, G.; Lepper, H.; Estler, C. *J. Chromatogr.* **1993**, *620*, 158.
14. Leemann, T.; Transom, C.; Dayer, P. *Life Sci.* **1993**, *52*, 29.
15. Wienkers, L.; Steenwyck, R.; Pearson, P. *Drug Metab. Dispos.* **1995**, *23*, 383.