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CVT-4325: a potent fatty acid oxidation inhibitor with favorable oral bioavailability

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Abstract—New inhibitors of palmitoyl-CoA oxidation are based on the introduction of nitrogen heterocycles in the 'Western Portion' of the molecule. SAR studies led to the discovery of CVT-4325 (shown), a potent FOXi (IC_{50} = 380 nM rat mitochondria) with favorable PK properties (F = 93%, $t_{1/2}$ = 13.6 h, dog).

The heart has two major sources of energy, fatty acids and glucose, that are both converted to acetyl-CoA units and subsequently utilized to produce ATP through the oxidative phosphorylation process.[1,2] Glucose can produce as much as 13% more ATP per mole of oxygen consumed than fatty acids.[2] Therefore, during ischemic disease where oxygen is limited, compounds that reduce the rate of fatty acid oxidation (i.e., partial fatty acid oxidation inhibitors, partial FOXi), and as a result, increase the rate of glucose oxidation (i.e., state dependent metabolic shift) are of potential therapeutic importance.[1–3]

Compound **1** was found to be an inhibitor of 1-[^{14}C]-palmitoyl-CoA oxidation (PalmCoA oxidation inhibitor) in mitochondria isolated from rat heart (IC_{50}: 2.5 μM),[4,5] and also, it was shown to have low metabolic stability with 21% of the parent compound remaining after 30 min incubation with human liver S9 preparations.[6]

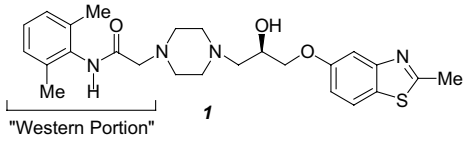

Figure 1. PalmCoA oxidation inhibitor **1**.

We conducted a study to investigate the structure–activity relationships (SAR) in the 'Western Portion' of our lead molecule, **1**, in order to optimize the potency with respect to palmCoA inhibition and metabolic stability (human liver S9, Table 1). We focused our efforts on finding a suitable bioisosteric replacement for the amide group by preparing various nitrogen heterocycles. Compounds with favorable palmCoA inhibition (IC_{50} < 500 nM) and metabolic stability (>40% remaining @ 30 min) were further evaluated in pharmacokinetic (PK) studies.

All of the compounds containing an amide surrogate in Table 1 were prepared by reacting the corresponding chloromethyl derivative **VII** with the previously described piperazine **3** as illustrated in Scheme 1.[5,7]

Keywords: CVT-4325; Metabolic shift; Fatty acid oxidation inhibitor.
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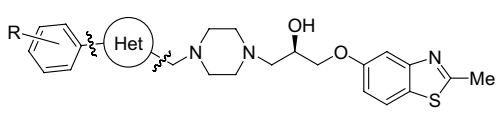
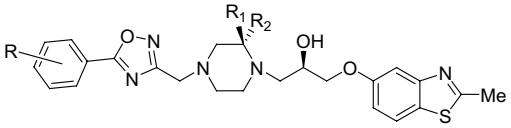
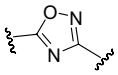
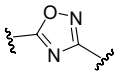
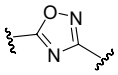
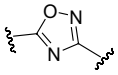
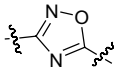
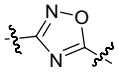
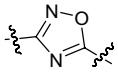
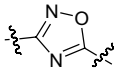
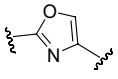
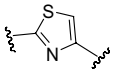
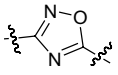
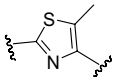
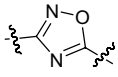
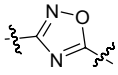
Table 1. Palmitoyl-CoA oxidation inhibition activity of **4**–**33**



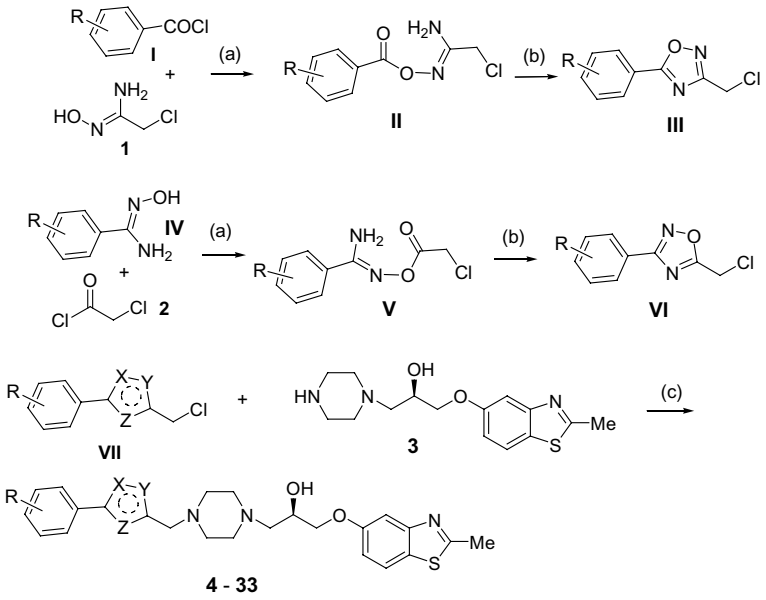


Compd #[a]	R	Heterocycle	PalmCoA IC_{50} (uM)[b]	Human liver S9 % @ 30 min[c]	Compd #[a]	R	R_1, R_2	PalmCoA IC_{50} (uM)[b]	Human liver S9 % @ 30 min[c]
4	4-^{t}Bu		**0.18**	18	**20**	3,4-Di-Cl	H, H	**0.55**	30
5	4-^{i}Pr		0.95	31	**21**	2,4-Di-Cl	H, H	**0.13**	33
6	4-Cl		2.27	48	**22**	4-CN	H, H	4.41	56
7	4-CF_3		**0.38**	74	**23**	4-OMe	H, H	1.53	39
8	4-^{t}Bu		0.57	39	**24**	4-OCF_3	H, H	1.71	—
9	4-^{i}Pr		1.23	21	**25**	4-F	H, H	1.53	50
10	4-Cl		6.80	66	**26**	3-Me	H, H	6.50	31
11	4-CF_3		3.63	72	**27**	3-Cl	H, H	4.12	38
12	4-^{t}Bu		1.10	25	**28**	3-F	H, H	13.4	42
13	4-Cl		8.50	34	**29**	3-CN	H, H	13.4	—
14	4-CF_3		4.80	49	**30**	4-CF_3	H, Me	**0.25**	43
15	4-CF_3		11.0	43	**31**	2,4-Di-Cl	H, Me	**0.12**	17
16	4-CF_3		1.30	40	**32**	2,4-Di-Cl 	H, Me	**0.18**	30
17	4-CF_3		1.50	40	**33**	4-CF_3	Me, Me	9.7	—
18	3,4-Di-Cl		1.56	36					
19	2,4-Di-Cl		1.14	29					

[a] All compounds were >95% pure by HPLC and characterized by ^{1}H NMR, MS, and LC/MS. Some compounds were further characterized by elemental analysis.

[b] Compounds were tested at six different concentrations, and IC_{50} values were calculated using nonlinear regression curve fitting in GraphPad (Prism 3.00 data shown are mean values ($n = 3$)).

[c] Percent of parent drug remaining after incubation with human liver S9 (2 mg/mL) in the presence of NADPH for 30 min. A standard was included in every assay for the liver S9 and the data was not used unless the standard was within ±5% of the historical mean.



Scheme 1. Reagents and conditions: (a) DIEA, CH_2Cl_2, rt, 24 h; (b) toluene, reflux, 24 h; (c) R-CH_2Cl, DIEA, EtOH, 80 °C, 24 h.

The 5-phenyl-1,2,4-oxadiazoles **III** and the 3-phenyl-1,2,4-oxadiazoles **VI** were prepared from the corresponding amide oximes and acid chlorides by acylation followed by condensation.[8,9] The required chloromethyl heterocycles **VII** to prepare the 2-phenyl-1,3,4-oxadiazole derivatives **12**–**14** (Table 1) were prepared from the corresponding acyl hydrazides and 2-chloro-1,1,1-trimethoxyethane as previously described.[10] Also, several of the chloromethyl heterocycles **VII** required to prepare the target compounds in Table 1 were commercially available (including **7**, **15**, **16**, and **17**). The 2-(*S*)-methyl piperazine derivatives (**30**–**32**) and 2,2-dimethylpiperazine derivative (**33**) were prepared starting with a regioselective Boc protection of the corresponding piperazines (i.e., 4-N-Boc) followed by epoxide opening and Boc deprotection to obtain the methylated analogs of **3**.[11] Then, the target compounds **30**–**33** were obtained through alkylation with the corresponding chloromethyl heterocycles **VII** as described above.

We chose nitrogen heterocycles as amide surrogates, since there is plenty of literature precedence. In particular, oxadiazoles have been used extensively as amide surrogates in the peptide mimetic area,[12–15] and even in the adenosine field, as a 5′-amide surrogate of 5′-*N*-ethylcarboxamide adenosine (NECA).[16] Based on previous SAR of the anilide series,[7] the *para* position of the 5-phenyl-1,2,4-oxadiazoles **4**–**7**, and the 3-phenyl-1,2,4-oxadiazoles **8**–**11** was substituted by either a lipophilic or electron withdrawing group (EWG). Although the palmCoA inhibition was favorable for derivatives containing a 4-*t*-butyl group, **4** and **8**, both derivatives had low metabolic stability (Table 1). Replacing the 4-*t*-butyl group with a smaller isobutyl group lowered the palmCoA inhibition (**5** and **9**). The 1,2,4-phenyloxadiazole analogs containing a *para*-EWG, either chloro (**6** and **10**) or trifluoromethyl (**7** and **11**) were found to be more metabolically stable (liver S9, Table 1). The *para*-trifluoromethyl 5-phenyl-1,2,4-oxadiazole **7** had the best combination of palmCoA inhibitory activity and metabolic stability (Table 1). A brief exploration of the SAR of the 2-phenyl-1,3,4-oxadiazole series with respect to *para*-*t*-butyl, *para*-chloro, and *para*-trifluoromethyl (**12**–**14**) demonstrated that this series is less active in the palmCoA assay than either 1,2,4-oxadiazole series (i.e., 3-phenyl and 5-phenyl). In general, within the oxadiazole series explored, the following trend was observed with respect to palmCoA inhibition: 5-phenyl-1,2,4-oxadiazole > 3-phenyl-1,2,4-oxadiazole > 2-phenyl-1,3,4-oxadiazole. Based on the palmCoA inhibitory activity and metabolic stability of **7**, additional heterocycles were explored with *p*-trifluoromethylphenyl groups, **15**–**17**; however, the 5-phenyl-1,2,4-oxadiazole remained the best amide surrogate. We next explored the two best oxadiazole series with either 3,4- or 2,4-dichlorophenyl substitution, **18** and **20** or **19** and **21**, respectively, and once again, the 5-phenyl-1,2,4-oxadiazole series afforded the best palmCoA inhibition. Unfortunately, **20** and **21** were not metabolically stable (Table 1). Further SAR on the 5-phenyl-1,2,4-oxadiazole series **22**–**30** demonstrates that a preference for *para* versus *meta* substitution with respect to palmCoA inhibition (**6** vs **27**, **22** vs **29**, **25** vs **28**). Previously within the anilide SAR,[5] a 2-(*S*)-methylpiperazine enhanced the inhibitory activity in the palmCoA assay, and this trend appears to hold in part for the 1,2,4 oxadiazole series as well (**30** > **7**, **32** > **19**, **31** = **21**). However, the 2,2-dimethylpiperazine analog of **1** (Fig. 1) was very active in the palmCoA assay (Structure not shown, IC_{50} = 110 nM),[6] and this trend did not hold for the 2,2-dimethylpiperazine analog **33** (Table 1). The 2-(*S*)-methylpiperazine derivative **30** had high palmCoA inhibitory activity with modest metabolic stability, and it was chosen for further PK analysis along with the lead **7** (CVT-4325), and the parent anilide **1**.

The PK properties of **1** in rats were not very favorable with respect to AUC (dose adjusted), $t_{1/2}$, and clearance;

Table 2. Comparison of pharmacokinetic properties of **1**, **7** [(R)-CVT-4325], and **30**

Compound (species)	Oral dose[a] MPK	%*F*	AUC (ng h/mL)[b]	$t_{1/2}$ (h)[c] IV	Plasma clearance (mL/min/kg)[c]
1 (rat)	2	19	30.5	1.4	54.5
7 (rat)	2	38	282	3.2	22.5
7 (dog)	4	93	1776	13.6	12.2
30 (rat)	2	29	253	4.0	19.7
30 (dog)	5	30	1093	65.0	4.6

[a] PO formulation: propylene glycol:0.1 N HCl:Tween 80:0.5% carboxycellulose = 20:5:0.4:74.6 (v:v:v:v). Final concentrations in formulation: 2–5 mg/mL.

[b] Dose adjusted oral AUC normalized to 1 mg/kg.

[c] IV formulation: propylene glycol:0.1 N HCl:saline water = 20:5:75 (v/v/v). Final concentration in formulation: 1 mg/mL (1 mg/kg dose).

and therefore, it was not further evaluated in dogs. The lead compounds **7** and **30** were evaluated in both rat and dog, and the results are shown in Table 2. Both amide surrogate compounds **7** and **30** have clearly superior PK profiles relative to anilide **1** based on comparison of AUC, $t_{1/2}$, and clearance in the rat. A closer comparison of **7** and **30** demonstrated that **7** has higher oral bioavailability in both rats and dogs. However, **30** has a low clearance and extremely long half-life in dogs. We further compared the rates of disappearance of **7** and **30** by liver microsomes from rats and dogs and confirmed that while metabolism rates of these two compounds were similar in rat liver microsomes, **30** was metabolically more stable than **7** in dog liver microsomes (74% and 62% parent @ 30 min, respectively). This small difference in the in vitro metabolism between **7** and **30** does not account for the observed decrease in plasma clearance and increase in oral bioavailability in dogs found with **30** that maybe attributed to many factors including a possible diminishment in *N*-dealkylation of the core piperazine ring, a known route of metabolism for ranolazine.[17]

In conclusion, the introduction of a *para*-trifluoromethyl-5-phenyl-1,2,4-oxadiazole, **7** (CVT-4325), as an amide surrogate resulted in both good palmCoA inhibition (IC_{50} = 380 nM) and a favorable PK profile (F = 93%, $t_{1/2}$ = 13.6 h, n = 3, dog). Additional pharmacological studies demonstrate that **7** produces a metabolic shift from fatty acids to glucose, and this finding is disclosed elsewhere.[18]

Acknowledgements

We would like to thank Dr. Brent Blackburn, and Dr. Luiz Belardinelli, for valuable input and discussions.

Supplementary data

Supplemental materials are available online that include the conditions of the PalmCoA assay. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2004.09.077.

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9. Piperazine **3** (24.4 g), 3-chloromethyl-5-(4-trifluoromethylphenyl)1,2,4-oxadiazole (20.8 g, Maybridge Plc, UK), ethanol (400 mL), and diisopropylethylamine (55.3 mL) were warmed at 70 °C for 16 h (TLC 3:1 hexanes/ethyl acetate). After concentration in vacuo, the reaction was diluted with water (500 mL), extracted with ethyl acetate (2 × 500 mL), and dried ($MgSO_4$). After concentration in vacuo (yellow powder obtained), trituration with minimal amounts of 3:1 mixture of hexanes and ethyl acetate resulted in a white powder, 31.5 g (74% yield) of **7** (CVT-4325). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.28 (d, 2H, J = 8.4 Hz); 7.79 (d, 2H, J = 8.0 Hz); 7.64 (d, 1H, J = 8.8 Hz); 7.42 (br s, 1H); 7.00 (dd, 1H, J = 8.4, 2.4 Hz); 4.30–4.20 (m, 1H); 4.09–4.02 (m, 2H); 3.83 (s, 2H); 3.50 (br s, 1H); 2.80 (s, 3H); 2.90–2.50 (m, 10H). MS (ESI+, *m*/*z*): 534.98.
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