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Novel inhibitors of fatty acid oxidation as potential metabolic modulators☆

Elfatih Elzein,[a,*] Kevin Shenk,[a] Prabha Ibrahim,[a] Tim Marquart,[a] Suresh Kerwar,[b] Stephanie Meyer,[b] Hiba Ahmed,[b] Dewan Zeng,[b] Nancy Chu,[c] Daniel Soohoo,[c] Shirley Wong,[c] Kwan Leung[c] and Jeff Zablocki[a]

[a]*Department of Bioorganic Chemistry, CV Therapeutics Inc., 3172 Porter Drive, Palo Alto, CA 94304, USA*
[b]*Department of Drug Research and Pharmacological Sciences, CV Therapeutics Inc., 3172 Porter Drive, Palo Alto, CA 94304, USA*
[c]*Department of Pre-clinical Development, CV Therapeutics Inc., 3172 Porter Drive, Palo Alto, CA 94304, USA*

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Abstract—We describe the synthesis of novel inhibitors of fatty acid oxidation as potential metabolic modulators for the treatment of stable angina. Replacement of the 2*H*-benzo[*d*]1,3-dioxolene ring system in our initial lead **3** with different benzthiazoles, benzoxazoles and introducing small alkyl substituents into the piperazine ring resulted in analogues with enhanced inhibitory activity against 1-^{14}[C]-palmitoyl-CoA oxidation in isolated rat heart mitochondria (**6**, $IC_{50} = 70$ nM; **25**, $IC_{50} = 23$ nM).

Ischemic heart disease (IHD) continues to be the leading cause of death in the United States.[1] Chronic stable angina, a constituent of IHD has been estimated to occur in at least 6.4 million Americans.[2] Angina is a result of the deficiency between myocardial oxygen supply and demand. The primary mechanism of action of current anti-anginal drugs (nitrates, β-adrenoceptor antagonists, calcium channel blockers) is improvement of myocardial oxygen balance between supply and demand by either an increase in coronary blood flow or a decrease in cardiac mechanical function, or both.[3,4] Thus, all three major classes of anti-anginal drugs exert their effects by altering hemodynamics. Pharmacological agents that act by improving the efficiency of transduction of biochemical energy to mechanical contractile work have been proposed as an adjunct or alternative to the more traditional hemodynamic therapies.[5] This new class of agents is referred to as metabolic modulators and aimed to decrease the rate of fatty acid oxidation by the heart and increase the oxidation of pyruvate derived from glucose and lactate.[6] Switching myocardial energy production from fatty acid oxidation to glucose oxidation results in a greater ATP yield for a given rate of myocardial oxygen consumption (up to 11% with complete switch from fat to carbohydrate).[7] This could clearly be important in itself under conditions of limited tissue oxygen supply as in angina. In addition, these agents may also decrease lactate and hydrogen ion production under low flow conditions.[8,9]

Ranolazine[10,11] (**1**) and trimetazidine[12,13] (**2**) are two examples of this new class of anti-anginal drugs. Both compounds are believed to exert their anti-anginal effects in part by shifting myocardial energy metabolism from fatty acids towards glucose (through inhibition of β oxidation of fatty acids) as the substrate for production of ATP.[14–16] Studies have shown that ranolazine partially inhibits β-oxidation of fatty acids in a dose-dependent manner.[17] Studies have also shown that a strong correlation exists between palmitoyl-CoA oxidation in isolated mitochondria and fatty acids and glucose oxidation in isolated hearts.[18] Therefore, inhibition of palmitoyl-CoA oxidation in isolated mitochondria is predictive of the effects on fatty acid and glucose oxidation in isolated hearts. In this communication, we describe the synthesis of new fatty acid oxidation inhibitors (as measured by inhibition of 1-^{14}[C]-palmitoyl-CoA oxidation in rat heart mitochondria)[17] as potential

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*Corresponding author. Tel.: +-1-650-384-8217; fax: +1-650-858-0390; e-mail: elfatih.elzein@cvt.com

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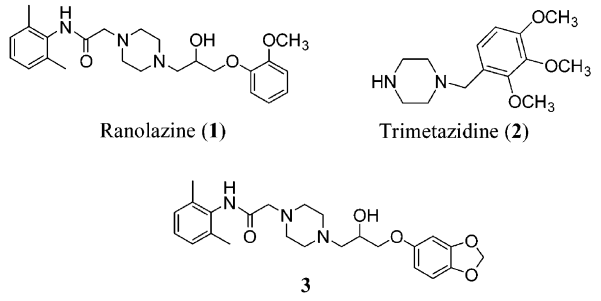
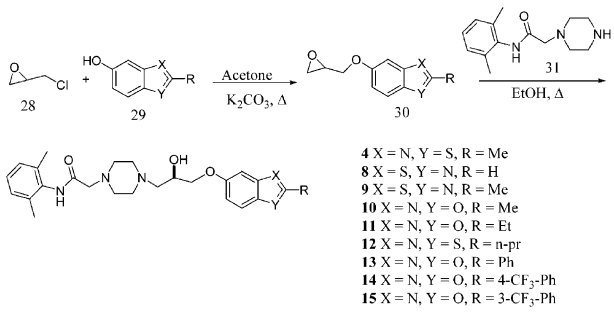


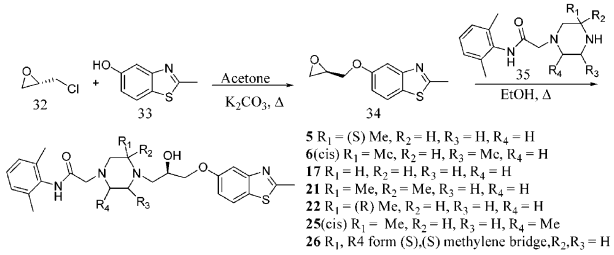
Figure 1.

metabolic modulators for the treatment of ischemic heart disease.

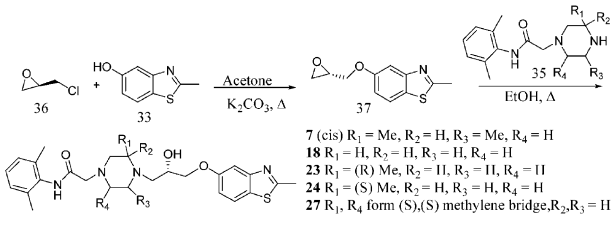
The synthesis of racemic analogues (**4**,**8**–**15**) is outlined in Scheme 1. Reaction of epichlorohydrin with 2-substituted benzoxazole or benzthiazole of the general formula **29** in acetone using K_2CO_3 as a base afforded the corresponding epoxide **30**. Opening of the epoxide with piperazine **31** was achieved by heating the mixture in ethanol. Studies have shown that reaction of (*S*) and/or (*R*)-epichlorohydrin with phenols using K_2CO_3 in acetone, predominantly proceeded via epoxide ring opening and led to inversion of assignment at the C-2 chiral center.[19–22] Procedures used in the synthesis of (*R*) and (*S*) epoxides **34** and **37** are shown in Schemes 2 and 3, respectively. Starting with the (*S*) and/or



Scheme 1.



Scheme 2.



Scheme 3.

(*R*)-epichlorohydrin **32** and **36**, 5-[((2*R*)oxiran-2-yl)methoxy]-2-methylbenzthiazole **34** and 5-[((2*S*)oxiran-2-yl)methoxy]-2-methylbenzthiazole **37** were obtained, respectively, in >95% ee using K_2CO_3 in acetone.[23] The different 2-substituted benzoxazoles and benzthiazoles were prepared following literature procedures.[24,25]

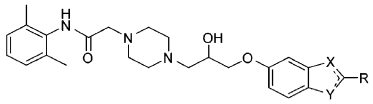
In our discovery paradigm, IC_{50}s were determined for compounds that show ≥50% inhibition of 1-^{14}[C]-palmitoyl-CoA (Palm CoA) oxidation in rat heart mitochondria at 100 μM. Compounds that display an IC_{50} of <50 μM were evaluated for their metabolic stability and CYP3A4 inhibition. Compounds that show ≥40% parent remaining after incubation with human liver microsomes (liver S-9) for 30 min, no CYP3A4 inhibition (IC_{50} > 30 μM) or have an IC_{50} in Palm CoA assay <1 μM were further evaluated for their oral bioavailability in rats. Our initial studies to define the minimum structural elements that were needed for greater Palm CoA oxidation inhibitory activity had led to the discovery of compound **3** (Fig. 1).[26] Compound **3** displayed 38% inhibition of Palm CoA oxidation at 100 μM. In addition, 80% of the parent compound was remaining after incubation with human liver microsomes for 30 min. However, in subsequent studies **3** was found to be an inhibitor of CYP3A4 (IC_{50} = 4.3 μM). Compound **3** also showed time dependent inhibition of CYP3A4 suggesting that **3** is a mechanism based inhibitor.[26] We then directed our efforts to the optimization of compound **3** with the idea of eliminating CYP3A4 inhibition, maintaining liver S-9 stability and improving its Palm CoA oxidation inhibitory activity.

Studies have shown that in a 2*H*-benzo[*d*]1,3-dioxolene ring system such as in **3**, the 1,3-dioxolene subunit is responsible for the potent mechanism based inhibition of CYP3A4.[27] In an attempt to find a suitable bioisostere for the 1,3-dioxolene subunit we replaced this subunit with different heteroaryls as shown in Table 1. Replacement of the 2*H*-benzo[*d*]1,3-dioxolene fused ring system in compound **3** with 2-methylbenzthiazole as in **4** resulted in at least a 10-fold increase in the inhibitory activity (IC_{50} = 10 μM) compared to **3**. Compound **4** showed no significant inhibition of the CYP3A4 isozyme (IC_{50} = > 30 μM), 50% parent remaining upon incubation with liver S-9 and good oral bioavailability and half-life in rats (Table 3). Extending the 2-alkyl group in **4** to ethyl and propyl groups resulted in compounds **11** and **12**, that displayed IC_{50}'s comparable to that of **4**; however, both analogues had less liver S-9 stability relative to **4** (<30%). The 2-methylbenzthiazole regioisomer **9** was completely devoid of inhibitory activity suggesting the importance of the sulfur group being *para* to the 5-ether linkage. For this class of 2-alkylbenzthiazole compounds, the 2-methyl substituent was found to be optimal for both inhibitory activity and liver S-9 stability. Compound **10**, a benzoxazole analogue of **4**, displayed a significant drop in Palm CoA oxidation inhibitory activity. However, introduction of a substituted phenyl group instead of the 2-methyl group in **10** restored the inhibitory activity and resulted in compounds (**13**–**15**) with IC_{50}'s comparable to that of **4**.

Among the three 2-phenylbenzoxazole compounds (**13**–**15**) only **14** showed liver S-9 stability that satisfied our criteria (40% parent remaining) and this was probably due to the presence of the trifluoromethyl group at the 4-position blocking that important site of metabolism. In this class of 2-substituted benzoxazole compounds and considering the limited number of compounds that were made, it seems that a 4-substituted aromatic ring might be optimal for Palm CoA oxidation inhibitory activity and liver S-9 stability.

The importance of the secondary hydroxyl group and its chirality was also investigated (Table 2). Removal of the hydroxyl group resulted in a significant loss in inhibitory activity (**16**) relative to **4**. This result suggests that the hydrogen bond donating or/and accepting ability of this hydroxyl group plays an important role in receptor-ligand interactions. The (*R*) configuration at the carbon bearing the hydroxyl group was required for the best enhancement of inhibitory activity (compound **17** versus compound **18**). However, the (*R*) enantiomer was

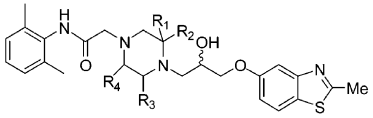
Table 1. Fatty acid oxidation inhibition and liver S-9 stability of heteroaryl analogues



Compd[a]	X	Y	R	% Inhibition[b] @ 100 μM	IC_{50} (μM)[c]	% Parent remaining after 30 min[d]
4	N	S	Me	88	10	50
8	S	N	H	3	—	—
9	S	N	Me	3	—	—
10	N	O	Me	34	—	—
11	N	S	Et	75	13	29
12	N	S	*n*-Pr	70	20	15
13	N	O	Ph	70	23	15
14	N	O	4-CF_3-Ph	89	8	40
15	N	O	3-CF_3-Ph	82	13	6

[a] All compounds were >95% pure by HPLC and characterized by ^{1}H NMR, MS, LC/MS. Some compounds were further characterized by elemental analysis.
[b] % Inhibition of 1-^{14}[C]-palmitoyl-CoA oxidation in rat heart mitochondria. 95% confidence limits were generally ±15% of the mean value.
[c] Concentration necessary to inhibit 50% of 1-^{14}[C]-palmitoyl-CoA oxidation in rat heart mitochondria, IC_{50} values are the mean of $n=3$ and were determined using the non-linear regression curve fitting by GraphPad (prism 3.0), experiments were carried out at 6 different concentrations.
[d] Amount of parent drug remaining after incubation with human liver microsomes for 30 min.

Table 2. Effect of piperazine ring substitution and hydroxyl group stereochemistry on inhibition of fatty acid oxidation and liver S-9 stability



Compd[a]	R_1	R_2	R_3	R_4	Hydroxyl group stereochemistry	IC_{50} (μM)[b] or % inhibition[c] @ 100 μM	% Parent remaining after 30 min[d]
4	H	H	H	H	racemic	10	50%
5	(*S*)-Me	H	H	H	(*R*)	0.2	<10%
6(*cis*)	Me	H	Me	H	(*R*)	0.07	21%
7(*cis*)	Me	H	Me	H	(*S*)	2.5	<30%
16	H	H	H	H	No hydroxyl	38%	<30%
17	H	H	H	H	(*R*)	4	<27%
18	H	H	H	H	(*S*)	47%	35%
20	Me	Me	H	H	racemic	0.45	<10%
21	Me	Me	H	H	(*R*)	0.11	<10%
22	(*R*)-Me	H	H	H	(*R*)	2	<10%
23	(*R*)-Me	H	H	H	(*S*)	10	<10%
24	(*S*)-Me	H	H	H	(*S*)	5	<30%
25(*trans*)	Me	H	H	Me	(*R*)	0.023	<10%
26	R_1, R_4 form CH_2 bridge	H	H	H	(*R*)	40%	—
27	R_1, R_4 form CH_2 bridge (*S*),(*S*)	H	H	H	(*S*)	26%	—

[a] All compounds were >95% pure by HPLC and characterized by ^{1}H NMR, MS, LC/MS. Some compounds were further characterized by elemental analysis.
[b] % Inhibition of 1-^{14}[C]-palmitoyl-CoA oxidation in rat heart mitochondria. 95% confidence limits were generally ±15% of the mean value.
[c] Concentration necessary to inhibit 50% of 1-^{14}[C]-palmitoyl-CoA oxidation in rat heart mitochondria, IC_{50} values are the mean of $n=3$ and were determined using the non-linear regression curve fitting by GraphPad (prism 3.0), experiments were carried out at 6 different concentrations.
[d] Amount of parent drug remaining after incubation with human liver microsomes for 30 min.

slightly less stable than the (*S*) enantiomer in the liver S-9 assay. Introduction of a 2-(*S*) methyl group in the piperazine ring of compound **17** (where the hydroxyl group has the (*R*) configuration) as in **5** (*S*,*R*-diastereomer) resulted in a substantial increase in Palm CoA oxidation inhibitory activity (IC_{50} = 200 nM). Compound **22** in which the carbon bearing the methyl group has the (*R*) configuration was 10-fold less active than **5**. Compound **23** where the methyl and the hydroxyl groups have the (*R*) and (*S*) configurations respectively, was at least 50-fold less active than **5**. This result is in agreement with our earlier finding that the (*R*) configuration at the carbon bearing the hydroxyl group is required for optimal inhibitory activity. In addition, the (*S*) configuration of the methyl group in the piperazine ring in conjunction with the (*R*) configuration of the hydroxyl group enhanced inhibitory activity. Introduction of a *gem*-dimethyl in **4** resulted in compound **20** that displayed a 22-fold increase in inhibitory activity relative to **4** (IC_{50} = 450 nM). Compound **21**, the (*R*) enantiomer of **20** showed 4-fold greater inhibitory activity (IC_{50} = 110 nM) compared to that of the racemate **20** and this is in accord with the finding that the (*R*) configuration of the hydroxyl group is optimal for enhanced inhibitory activity. Introduction of a 2,6-dimethyl group into the piperazine ring as in **6** yielded our second most active compound with an IC_{50} of 70 nM. As anticipated, **7** (where the hydroxyl group has the *S*-configuration) was less active than **6**. Compound **25**, the 2,5-dimethyl analogue was our most active compound with an IC_{50} of 23 nM. However, compound **26**, a constrained analogue of **25** showed weak inhibitory activity compared to **25**.

In general, compounds that incorporate at least one methyl group in the piperazine ring displayed significant inhibitory activity superior to those of unsubstituted piperazine analogues. Taking into account the high inhibitory activity of the methyl substituted piperazine analogues **5** and **6**, it is hypothesized that the methyl group could impart an orientation of the piperazine ring that is favorable for binding to the receptor. However, all active compounds with a methyl-substituted piperazine ring (e.g., **6**,**5**,**21**) showed reduced liver S-9 stability (Table 2) and decreased oral drug exposure in rats (Table 3). Significant improvement in the metabolic stability and oral bioavailability of these substituted piperazine analogues was achieved via isosteric replacement of the metabolically labile amide moiety (e.g., in compound **5**) with different 1,2,4-oxadiazoles as amide bioisosteres.[28] Detailed SAR that led to improvement in metabolic stability and oral bioavailability of these molecules and their effect on cardiac efficiency during ischemia will be reported in subsequent communication.

Table 3. Preliminary pharmacokinetic properties of selected palm CoA oxidation inhibitors in rats (values are average of $n = 3$)

Compd	Oral dose (Mg/Kg)	% F	AUC ng hr/mL	$t_{1/2}$ (h)
4	13	45	1256	3
5	9.7	5	278	6.2
6	11.2	11	128	3.5
21	10.5	13	265	4.2

In summary, the structure–activity relationships of our lead **3** was investigated. Replacement of the 2*H*-benzo[*d*]1,3-dioxolene ring in compound **3** with the 2-methylbenzthiazole scaffold resulted in a considerable enhancement in fatty acid oxidation inhibitory activity, significant reduction in CYP 3A4 inhibition and good half life and oral bioavailability in rats (compound **4**). Substantial improvement in the inhibitory activity was accomplished through introduction of a methyl group into the piperazine ring of **4**. Consequently, we have succeeded in the discovery of highly active inhibitors of fatty acid oxidation (**6**, IC_{50} = 70 nM; **25**, IC_{50} = 23 nM).

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Compound **3** was found to be a mechanism-based inhibitor of CYP3A4. It inhibits testosterone 6β-hydroxylase activity in NADPH-and time-dependent manner and its potency is similar to that of troleandomycin, a known positive mechanism based inhibitor of CYP3A4.
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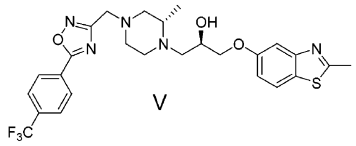

V