Secondary Amides of (*R*)-3,3,3-Trifluoro-2-hydroxy-2-methylpropionic Acid as Inhibitors of Pyruvate Dehydrogenase Kinase

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N′-Methyl-*N*-(4-*tert*-butyl-1,2,5,6-tetrahydropyridine)thiourea, SDZ048-619 (**1**), is a modest inhibitor (IC_{50} = 180 μM) of pyruvate dehydrogenase kinase (PDHK). In an optimization of the *N*-methylcarbothioamide moiety of **1**, it was discovered that amides with a small acyl group, in particular appropriately substituted amides of (*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid, are inhibitors of PDHK. Utilizing this acyl moiety, herein is reported the rationale leading to the optimization of a series of acylated piperazine derivatives. Methyl substitution of the piperazine at the 2- and 5-positions (with *S* and *R* absolute stereochemistry) markedly increased the potency of the lead compound (>1000-fold). Oral bioavailability of the compounds in this series is good and is optimal (as measured by AUC) when the 4-position of the piperazine is substituted with an electron-poor benzoyl moiety. (+)-1-*N*-[2,5-(*S*,*R*)-Dimethyl-4-*N*-(4-cyanobenzoyl)piperazine]-(*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (**14e**) inhibits PDHK in the primary enzymatic assay with an IC_{50} of 16 ± 2 nM, enhances the oxidation of [^{14}C]lactate into $^{14}CO_2$ in human fibroblasts with an EC_{50} of 57 ± 13 nM, diminishes lactate significantly 2.5 h post-oral-dose at doses as low as 1 μmol/kg, and increases the ex vivo activity of PDH in muscle, liver, and fat tissues in normal Sprague−Dawley rats. These PDHK inhibitors, however, do not lower glucose in diabetic animal models.

Introduction

The activity of the pyruvate dehydrogenase (PDH) complex is lower during conditions of reduced oxidative glucose metabolism such as obesity, starvation, and diabetes and in patients with congenital lactic acidosis.[1−6] The PDH complex catalyzes the decarboxylation of pyruvate to acetyl-CoA.[7] The activity of the PDH complex is primarily regulated via reversible phosphorylation. ATP-dependent phosphorylation of a specific E1 serine residue by four isozymes of pyruvate dehydrogenase kinases (PDHKs)[8−10] leads to inactivation of the complex. Dephosphorylation of the serine residue by pyruvate dehydrogenase phosphatases reactivates the complex.[11] High intramitochondrial concentrations of acetyl-CoA, which can be formed from excessive oxidation of free fatty acids, markedly increase the activity of the PDHK. It has been proposed that the reversible acetylation of the lipoamide of the E2 subunit of the PDH complex, which reversibly binds to PDHK, is responsible for this end product activation of PDHK.[12] This is consistent with the Randle hypothesis, which states that the oxidations of free fatty acids and glucose are related in a reciprocal manner.[13] Activation of the PDH complex via inhibition of PDHK would be expected to result in increased oxidation of pyruvate in muscle and fat and a concomitant decrease in the conversion of pyruvate to the gluconeogenic substrates lactate and alanine (see Figure 1).

Indeed, in early clinical studies, oral administration of sodium dichloroacetate (DCA), a known inhibitor of PDHKs, to type 2 diabetic patients lowered fasting plasma lactate, alanine, and glucose levels.[14−16] Although infusion of DCA lowered plasma lactate and alanine levels in healthy volunteers, no hypoglycemic effect was observed.[17] DCA has proven efficacy as a therapy for diabetes,[14−16] ischemia,[18] endotoxic shock,[19] hemorrhagic shock,[20] lactic acidosis,[21] and cardiac insufficiency.[22,23] However, DCA cannot be used in long-term treatment due to toxicity. The toxic effects presented by DCA are neuropathic effects, cataract formation, and testicular degeneration.[24−27] The neuropathy caused by DCA is exhibited primarily by reversible limb motor weakness and demyelination of cerebral and cerebellar white matter. The incidence of limb weakness in rats receiving 1.1 g/kg/day for 7 weeks is ∼6%. The toxic effects of DCA have been attributed in part to the accumulation of its main metabolite, oxalic acid. However, compounds with halides in the α-position to a carbonyl are known to exhibit toxic effects.[28,29] Nevertheless, PDHK inhibition may be a therapeutic approach to the treatment of type 2 diabetes, ischemia,

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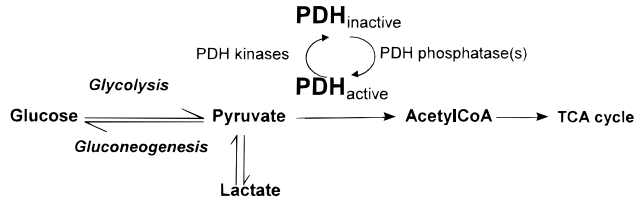


Figure 1. Rationale for PDHK inhibition. Inhibition of PDHK should enhance oxidative glycolysis in the muscle and decrease gluconeogenesis in the liver, thereby aiding in the restoration of euglycemia.

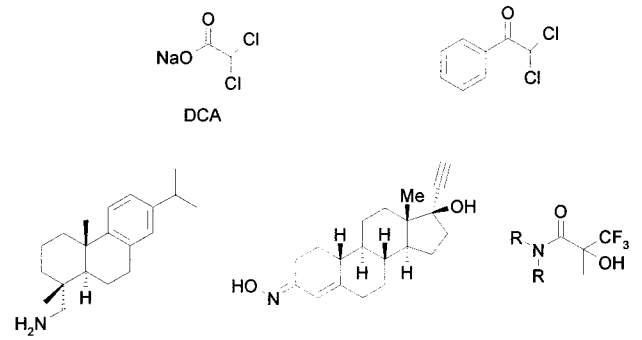
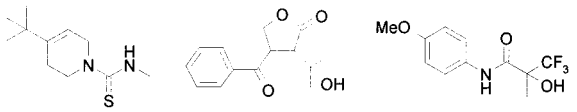


Figure 2. Inhibitors of PDHK.



1 **2** **3**

Figure 3. Initial leads.

lactic acidosis, and other indications with reduced capacity for the oxidative disposal of lactate and pyruvate.

Herein is reported a program which resulted in structurally novel, orally active inhibitors of the PDHK. Until our recent publications concerning di- and triterpenes, and a few of the amides elaborated upon in this paper (see Figure 2),[30,31] no compounds other than α,α-dihalogenated carbonyl compounds were known to inhibit PDHK.[32]

While the programming and logistics of screening compounds through our primary enzymatic assay[33,34] in a high-throughput mode on a robotic platform was being optimized, it was imperative to carefully select compounds for screening which would maximize diversity in the test set. To achieve this goal, we utilized a nonhierarchical clustering strategy based upon 2-D parameters distributed by MDL MACCS software in a manner similar to that reported by Martin et al.[35] Of the 200 randomly chosen compounds which were dissimilar to each other by a criteria of 60% SSS, two compounds, SDZ048-619 (**1**) and SDZ060-011 **(2)** (see Figure 3), presented very modest but interesting inhibition of PDHK. Subsequent screening identified the PDHK inhibitor SDZ225-066 **(3)**. On the basis of these data, a program to investigate the optimization of the six-membered ring and the acyl moiety of **1** was initiated in the quest for orally active PDHK inhibitors.

Chemistry

The desired compounds were prepared by a variety of methods, as outlined in Scheme 1. Conjugation of 4-substituted piperidines, 2-chloroaniline, cyclohexylamine, and 2-adamantylamine with the appropriate acyl chloride, sulfonyl chloride, or iso(thio)cyanate in the presence (method A) or absence (method B) of an appropriate base afforded the amides, sulfonamides, and (thio)ureas in excellent yields (see Table 1).

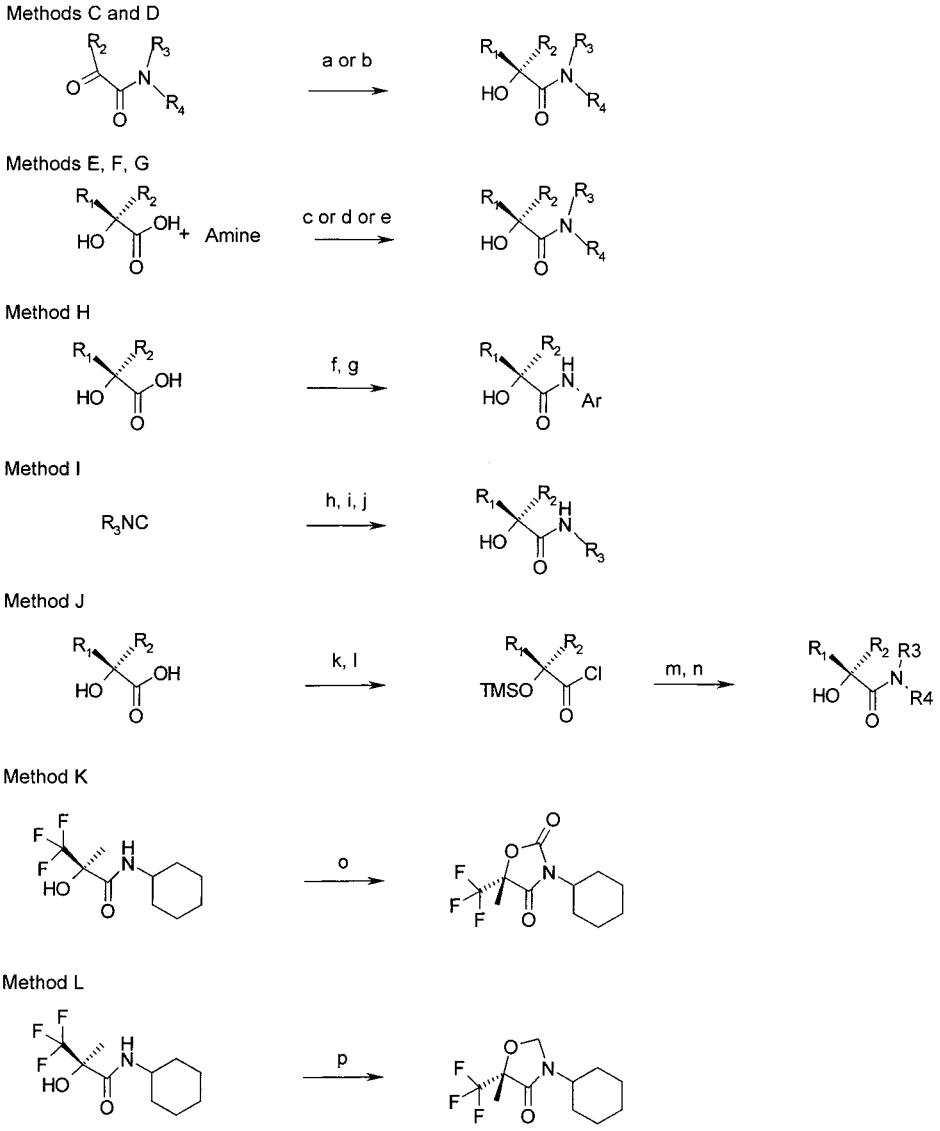
Our efforts soon became focused upon the α-hydroxyamides of substituted piperidines/piperazines (see Tables 1 and 2). The requisite amides of α-hydroxycarboxylic acids were synthesized via a number of methods: for example, by the addition of hydrides or Grignard reagents to pyruvamides (methods C and D). Even though the chemical scope of these reactions is very versatile, the choice of the medicinal targets was quickly limited (discussed below) to hydroxamides prepared containing small substituents (i.e., Me, CF_3, and hydrogen). As a result, it was more efficient to couple the few intact α-hydroxy acids to the appropriate amines. In our initial work, the coupling agents CDI (method E), DCC/HOBt (method G), and (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP; method F) were utilized for this purpose;[36] however, the yields were low and the couplings were generally only effective with cyclic secondary amines and saturated primary amines. Utilizing the procedure of Morris et al.,[37] anilides of α-hydroxy acids could be produced via treatment of the carboxylic acid with thionyl chloride (method H), followed by addition of the aniline.[38] The method is only effective with anilines, resulting in yields of 30−90% (anilines with electron-donating substituents afforded higher yields). Primary α-hydroxyamides were also produced from isocyanides and ketones via the procedure of Seebach et al. (method I).[39]

A modification of Kelly's procedure was the most general and effective synthetic method for α-hydroxyamides (method J).[40] In short, the acyl halide was prepared by first treating the α-hydroxycarboxylic acid with bis(trimethylsilyl)urea in CH_2Cl_2. After filtration to remove the urea byproduct, the bissilylated acid is converted to the acyl halide by treatment with oxalyl chloride in the presence of a catalytic amount of DMF. In the case of (*S*)-3,3,3-trifluoro-2-(trimethylsiloxy)-2-methylpropionyl chloride, produced from (*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid, the acid chloride can be stored for weeks at room temperature as the crude reaction mixture and utilized as such in aliquots with no discernible detrimental effect on the coupling yields. The crude α-siloxyamides are effectively desilylated by methanolic HCl. The couplings of the acyl chloride with the amines in this report was either effected with Et_3N in CH_2Cl_2 or with a modification of polymer-supported quench methodology reported by Hodges and Booth.[41] Although the yields of the couplings of bis(trifluoromethyl)-2-(trimethylsiloxy)acetyl chloride to amines were low (e.g., crude yield of **7f** was 35%), method J was the only procedure to afford product.

Based upon the emerging structure−activity relationships described below, it became desirable to prepare a variety of 3-substituted and 2,5-disubstituted piperazines. The synthetic routes are outlined in Scheme 2.

Retrosynthetically, the obvious route to the 3-methyl-substituted piperazines **11a**−**p** was to regioselectively monoacylate (*R*)-2-substituted piperazines with (*S*)-3,3,3-trifluoro-2-(trimethylsiloxy)-2-methylpropionyl chlo-

Scheme 1. Methods of Synthesis of PDHK Inhibitors[a]



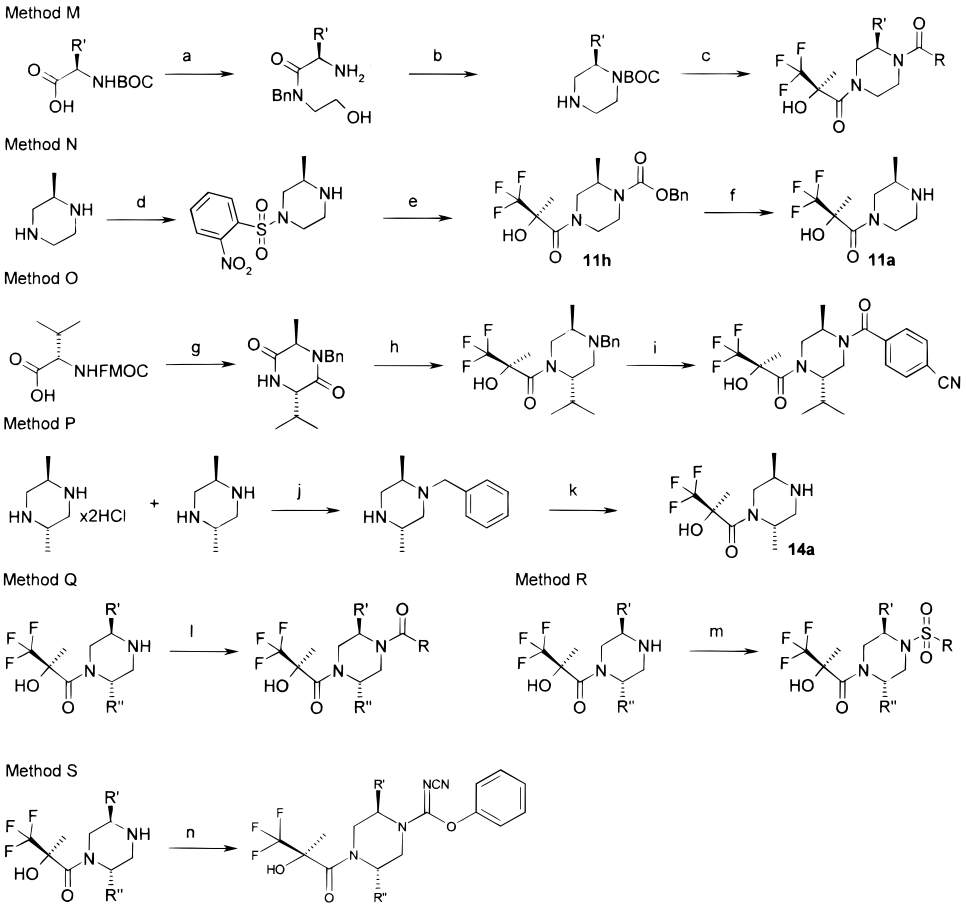
[a] Conditions: (a) MeMgBr, THF (Method C); (b) $NaBH_4$, EtOH (Method D); (c) CDI, CH_2Cl_2 (Method E); (d) PyBOP, *N*-methyl morpholine, CH_2Cl_2 (Method F); (e) DCC, HOBt (Method G); (f) $SOCl_2$, DMA; (g) $ArNH_2$; (h) $TiCl_4$, CH_2Cl_2; (i) R_1COR_2; (j) aq HCl; (k) 1,3-bis(trimethylsilylurea), CH_2Cl_2; (l) $(COCl)_2$, CH_2Cl_2, cat. DMF; (m) Et_3N, CH_2Cl_2, amine; (n) aq HCl, MeOH; (o) CDI, CH_2Cl_2; (p) $(CH_2O)_n$, toluene, *p*-toluenesulfonic acid.

ride. However, monoacetylation of piperazines is difficult,[42] and varied attempts to monoacetylate piperazines with (*S*)-3,3,3-trifluoro-2-(trimethylsiloxy)-2-methylpropionyl chloride failed. For systematic monosubstitution of piperazine analogues at the 3-position with different substituents, the method of Jacobsen et al. was employed.[43] It should be noted that some racemization of the chiral carbon was observed in this method (see method M; a final product with ~90% enantiomeric excess was typical in our hands). This impurity could be eliminated via recrystallization and/or chromatographic separation of the desired amide from the separable diastereomeric impurity produced in the subsequent coupling with (*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid.

A second route (see method N) toward 3-methylpiperazine analogues, which proved to be more practical on a large scale, took advantage of the report that 2-substituted piperazines can be monosulfonylated.[44] Employing the protecting moiety developed by Fukayama et al.,[45] (*R*)-2-methylpiperazine was regioselectively monosulfonylated with 2-nitrobenzenesulfonyl chloride. Consequently, large quantities of **11h** from (*R*)-2-methylpiperazine were obtained in excellent overall yield (51%) and enantiomeric purity (>99%). Reductive cleavage of the carbobenzyloxy moiety gave **11a**, which was further functionalized via methods Q, R, and S to afford **11b**−**p**.

2,5-Disubstituted piperazines were synthesized in a manner similar to method M via the diketopiperazones (method O). When synthetic work focused on derivatives of 1-*N*-[(*R*,*S*)-2,5-dimethylpiperazine]-(*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide, the parent compound **14a** (see method P) was conveniently prepared from achiral *trans*-2,5-dimethylpiperazine by monobenzylating a 1:1 mixture of the bishydrochloride salt and the free base in absolute ethanol in a modification of Craig and Young's procedure.[46] The 1-benzyl-*trans*-2,5-di-

Scheme 2. Synthesis of Substituted Piperazine PDHK Inhibitors[a]



[a] Conditions: (a) 1. CDI, CH_2Cl_2; 2. *N*-Benzylethanolamine; 3. TFA; (b) 1. DEAD, PPh_3, THF; 2. LAH, THF; 3. BOC_2O; 4. $Pd(OH)_2$, H_2, EtOH; (c) 1. (*S*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionyl chloride, base, CH_2Cl_2; 2. aq HCl, MeOH; 3. TFA; 4. base, CH_2Cl_2, RCOCl; (d) 2-(NO_2)-$C_6H_4SO_2Cl$, K_2CO_3, aq acetone; (e) 1. CBZCl, K_2CO_3, aq acetone; 2. PhSH, K_2CO_3, DMF; 3. Et_3N, CH_2Cl_2, (*S*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionyl chloride; 4. aq HCl, MeOH; (f) Pd/C, H_2, EtOH; (g) 1. $(COCl)_2$, CH_2Cl_2, cat. DMF; 2. *N*-Bn-L-alanine methyl ester, CH_2Cl_2, Et_3N; 3. NH_3, MeOH; (h) 1. $LiAlH_4$, THF; 2. (*S*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionyl chloride, base, CH_2Cl_2; 3. aq HCl, MeOH; (i) 1. Pd/C, H_2, EtOH; 2. Et_3N, CH_2Cl_2, 4-CN-C_4H_4COCl; (j) EtOH, BnCl; (k) 1. 2 eq (-)-tartaric acid, H_2O, recrystallize; 2. (*S*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionyl chloride, base, CH_2Cl_2; 3. aq HCl, MeOH; 4. Pd/C, H_2, EtOH. (l) RCOCl, base, CH_2Cl_2; (m) RSO_2Cl, base, CH_2Cl_2; (n) PhOC(NCN)OPh, THF.

methylpiperazine was efficiently resolved as the bistartrate salt in a single recrystallization from methanol to afford a 90% recovery of material of greater than 90% enantiomeric excess. A second crystallization afforded the enantiomerically pure amine. Acylation of the salt as above produced **14b**, followed by reduction over Pd in ethanolic HCl to afford **14a**, which was further functionalized via methods Q, R, and S to **14c**−**l**.[47]

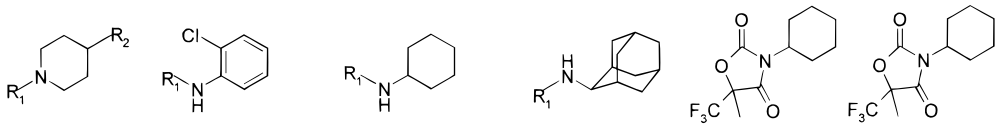
Results and Discussion

The thiourea **1** (IC_{50} = 180 μM) was found during our initial screening of 200 dissimilar compounds of the Novartis compound library, which, due to our previous interest in HDL elevating agents,[48] contains a significant number of thioureas of functionalized piperazines. Upon testing these compounds, it immediately became apparent that a 4-substituent of the piperidine was necessary for compounds to exhibit modest inhibition of PDHK in the primary assay (i.e., compare **1** and **4b** to **4x** and **4y**) and that the *N*-methylthiourea of **1** could be replaced with an acetyl (i.e., **4d**) or *N*-methylurea (i.e., **4b**) but not larger urea groups (i.e., compare **4b** with **4p** and **4q**) nor larger acyl groups (i.e., compare **4i** and **6a(*R*)** with **4r** and **6b**). *N*-Methylureas and acetamides of primary and noncyclic secondary amines were not inhibitory of PDHK (tested to 1 mM; data not shown).

The dichloroacetamide analogue **4i** (IC_{50} = 179 nM) was the most potent amide of 4-benzylpiperidine tested. Compound **4i** increased lactate oxidation in the cellular assay (EC_{50} = 1.12 μM; assay discussed below). However, as numerous toxicities have been reported with substances containing an α,α-dihalogenated carbonyl,[24−29] a search to find a suitable replacement for it was begun. Moieties which were considered for replacement of the chlorides of **4i** were fluoro, hydroxyl, trifluoromethyl, and methyl. The methyl and hydroxyl moieties are approximately isosteric, and the hydroxyl moiety is electron-withdrawing (but has the additional capability of two hydrogen bonds). The initial synthetic analogues **4f**, **4g**, and perfluorinated compounds (i.e., **4l**) were >100-fold less potent than **4i**.

The identification of **3** (IC_{50} = 23 μM, EC_{50} = 43 μM) as an inhibitor of PDHK in the primary enzymatic assay was exciting. Amides of (*S*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid had been demonstrated to be orally bioavailable and are being investigated for the indication of urinary incontinence.[49] The 3,3,3-trifluoro-

Table 1. Physical, in Vitro, and in Vivo Properties of Amides (Acyl Optimization Study)



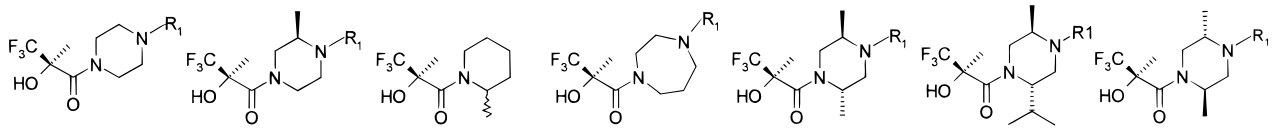
4a-v **5a-g** **6a-b** **7a-h** **8** **9**

entry	R_1	R_2	mp (°C)	empirical formula[a]	% yield (method)	IC_{50} (μM)[b]	EC_{50} (μM)[c]	lactate[d] (% of control, μmol/kg)
DCA[e]						>1000	130 ± 60	70%, 1 mmol/kg
4a	CSNHMe	Bn	101−102	$C_{14}H_{20}N_2S$	97 (A)	560 ± 240		
4b	CONHMe	Bn	oil	$C_{14}H_{20}N_2O$	79 (A)	22.4 ± 3.1	inactive	
4c	$CONMe_2$	Bn	oil	$C_{15}H_{22}N_2O$	85 (B)	416 ± 88		
4d	COMe	Bn	oil	$C_{14}H_{19}NO$[f]	92 (B)	74 ± 19	inactive	
4e	CO*t*Bu	Bn	84−85	$C_{17}H_{25}NO$	67 (B)	282 ± 38	inactive	
4f	$COC(OH)(CH_3)_2$	Bn	177	$C_{16}H_{23}NO_2$	54 (C)	150 ± 25		
4g	COCH(OH)Me	Bn	73−75	$C_{15}H_{21}NO_2$[g]	78 (D)	310 ± 31		
4h	COC(OH)(*c*Pr)	Bn	99−100	$C_{16}H_{21}NO_2$	42 (G)	260 ± 60		
4i	$COCHCl_2$	Bn	62−63	$C_{14}H_{17}NOCl_2$	71 (B)	0.179 ± 0.039	1.12 ± 0.31	not active, 300 μmol/kg
4j	$COCHClCH_3$	Bn	65−66	$C_{15}H_{20}NOCl$	75 (B)	2.6 ± 0.80		
4k	$COCCl_3$	Bn	39−40	$C_{14}H_{16}NOCl_3$	94 (B)	30 ± 3.3		
4l	$COCF_3$	Bn	oil	$C_{14}H_{16}NOF_3$	92 (B)	inactive		
4m	SO_2NHMe	Bn	oil	$C_{13}H_{20}N_2O_2S$	39 (B)	inactive		
4n	COOMe	Bn	40−42	$C_{14}H_{19}NO_2$	93 (B)	~1000		
4p	CONH(*p*-ClPh)	Bn	188	$C_{19}H_{21}N_2OCl$	90 (A)	inactive		
4q	CONH*i*Pr	Bn	93	$C_{16}H_{24}N_2O$	79 (A)	inactive		
4r	$COCClCH_2iPr$	Bn	oil	$C_{18}H_{26}NOCl$	47 (B)	inactive		
4s[h]	$COC(CF_3)(OH)Me$	Bn	60−62	$C_{16}H_{20}NO_2F_3$	21 (E)	2.16 ± 0.51	29.0 ± 5.5	not active, 300 μmol/kg
4t(*R*)	*R*-$COC(CF_3)(OH)Me$	Pr	81−83	$C_{12}H_{20}NO_2F_3$	37 (F)	3.1 ± 0.5		
4t(*S*)[j]	*S*-$COC(CF_3)(OH)Me$	Pr	82−83	$C_{12}H_{20}NO_2F_3$	43 (F)	75 ± 1.1		
4u	$COC(CF_3)(OMe)Me$	Pr	oil	$C_{13}H_{22}NO_2F_3$	75 (B)	inactive		
4v[h]	$COC(CF_3)(OH)Me$	H	68−69	$C_9H_{14}NO_2F_3$	24 (F)	23.9 ± 3.2		
4x	$COCH_3$	H	oil	$C_7H_{13}NO$	86 (B)	inactive		
4y	CONHMe	H	oil	$C_7H_{14}N_2O$	87 (A)	inactive		
5a(*R*)	*R*-$COC(CF_3)(OH)Me$		112−114	$C_{10}H_9NO_2ClF_3$	43 (F)	0.300 ± 0.028	5.4 ± 0.39	not active, 300 μmol/kg
5a(*S*)[j]	*S*-$COC(CF_3)(OH)Me$		113−114	$C_{10}H_9NO_2ClF_3$	64 (F)	9.8 ± 0.57		
5b	$COC(OH)(CH_3)_2$		94−96	$C_{10}H_{12}NO_2Cl$	48 (G)	5.0 ± 0.80		
5c	$COC(CF_3)(OMe)Me$		oil	$C_{11}H_{11}NO_2ClF_3$	74 (B)	inactive		
5d	$COC(CF_3)(OH)Et$		95−96	$C_{11}H_{11}NO_2ClF_3$	36 (G)	20 ± 2.3		
5e	SO_2NHMe		oil	$C_7H_9N_2O_2SCl$	48 (B)	inactive		
5f	COC(Et)(Me)OH		oil	$C_{11}H_{14}NO_2ClF_3$	55 (G)	23 ± 2.0		
5g	$COCHCl_2$		107−108	$C_8H_6NOCl_3$	90 (B)	47 ± 7.7		
6a(*R*)	*R*-$COC(CF_3)(OH)Me$		79−80	$C_{10}H_{16}NO_2F_3$	73 (F)	6.2 ± 0.51	*i*	
6a(*S*)[j]	*S*-$COC(CF_3)(OH)Me$		83−84	$C_{10}H_{16}NO_2F_3$	58 (F)	200 ± 24		
6b	$COC(CF_3)(OPh)Me$		117−118	$C_{15}H_{18}NO_2F_3$	46 (I)	inactive		
7a(*R*)	*R*-$COC(CF_3)(OH)Me$		152−153	$C_{14}H_{20}NO_2F_3$	80 (F)	0.30 ± 0.029	*i*	not active, 300 μmol/kg
7a(*S*)[j]	*S*-$COC(CF_3)(OH)Me$		152−154	$C_{14}H_{20}NO_2F_3$	65 (F)	11 ± 0.56		
7b	$COC(OH)(CH_3)_2$		102−104	$C_{14}H_{23}NO_2$	90 (F)	10 ± 0.65		
7c	$COC(CF_3)(OMe)Me$		61−62	$C_{15}H_{22}NO_2F_3$	82 (B)	inactive		
7d	$COC(CF_3)(OH)Et$		165−166	$C_{15}H_{22}NO_2F_3$	71 (F)	14 ± 0.94		
7e	SO_2NHMe		134−136	$C_{11}H_{20}N_2O_2S$	61 (E)	inactive		
7f	$COC(OH)(CF_3)_2$		128−129	$C_{14}H_{17}NO_2F_6$	9 (J)	17 ± 2.0		
7g	COCOMe		87−90	$C_{13}H_{19}NO_2$	5 (G)	inactive		
7h	COC(OH)(*c*Pr)		119−120	$C_{14}H_{21}NO_2$	60 (G)	15 ± 1.6		
8			67−69	$C_{11}H_{14}NO_3F_3$	83 (K)	inactive		
9			50−52	$C_{11}H_{16}NO_2F_3$[k]	17 (L)	inactive		

[a] Analytical results (C, H, N) were within ±0.4% of the theoretical value unless otherwise noted. [b] IC_{50} (μM ± standard error) in primary enzymatic assay of PDHK inhibition (ref 34). [c] EC_{50} (μM ± standard error) in cellular assay of increased oxidation of lactate (ref 60).[d] In vivo study in normal Sprague−Dawley rats (n = 6/group); animals were orally dosed (μmol/kg) after a 24-h fast. Lactate expressed as percent of control, 2 h post-dose. [e] Sodium dichloroacetate. [f] C: calcd 77.38; found 76.79. [g] C, H: calcd 73.53, 8.87; found 74.30, 9.30. [h] Prepared with racemic 3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid. [i] Increased lactate conversion to CO_2 6−10-fold when dosed at 10× the IC_{50} (ref 61). [j] Synthesized from (*S*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid of 96% ee. [k] C: calcd 52.58; found 53.12.

2-hydroxy-2-methylpropionic amide of 4-benzylpiperidine, **4s**, was found to be a modest (IC_{50} = 2.16 μM) but promising inhibitor of PDHK. Generally, (*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamides are modest inhibitors of PDHK (i.e., **4s**, **5a(*R*)**, **6a(*R*)**, and **7a(*R*)** IC_{50} values from 0.30 to 6.2 μM). In all amides generated from 3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid, the (*R*)-enantiomer is substantially (~30-fold) more potent than the (*S*)-enantiomer. Similar to the above-mentioned consequences of increasing the size of the dichloroacetyl of **4i** or the urea of **4b**, increasing the size of the 3,3,3-trifluoro-2-hydroxy-2-methylpropionic moiety in these PDHK inhibitors afforded significantly less potent inhibitors. For example, the replacement of the methyl with an ethyl afforded an approximate 10-fold decrease in potency (compare **5a(*R*)** and **7a(*R*)** with **5d** and **7d**). Also, substitution of the hydroxyl moiety with methoxy or modification of the OH via cyclization of the NH of the amide and the hydroxyl moiety with either carbonyldiimidazole or formaldehyde afforded compounds which did not inhibit PDHK in the functional assay (compare **6a** with **6b** or **8** or **9**, compare **5a** with

Table 2. Physical, in Vitro, and in Vivo Properties of Piperazine Analogues



10a-j **11a-p** **12** **13a-c** **14a-j** **15** **16a,b**

entry	R_1	mp (°C)	empirical formula[a]	% yield (method)	IC_{50} (μM)[b]	EC_{50} (μM)[c]	lactate[d] (% of control, μmol/kg)
10a	H	148−150	$C_8H_{13}N_2O_2F_3$	91 (N)	inactive		
10b	Bn	oil	$C_{15}H_{19}N_2O_2F_3$	98 (J)	19.9 ± 1.5		
10c[e]	PhCO	156−158	$C_{19}H_{19}N_2O_3F_3$	69 (J)	31.7 ± 2.0		
10d[e]	$PhSO_2$	147−149	$C_{14}H_{17}N_2O_4SF_3$	50 (R)	5.56 ± 0.73		
10e	4-NC(C_6H_4)CO	173−174	$C_{16}H_{16}N_3O_3F_3$	65 (Q)	6.7 ± 0.97		
10f[e]	PhOC(NCN)	oil	$C_{16}H_{17}N_4O_3F_3$	56 (S)	6.0 ± 1.5		
10g	4-NO_2-2-pyridyl	137−138	$C_{13}H_{15}N_4O_4F_3$	69 (J)	2.84 ± 0.25		
10h[e]	1-naphthoyl	95	$C_{19}H_{19}N_2O_3F_3$	69 (Q)	5.02 ± 0.59		
10i[e]	Ph	97−98	$C_{14}H_{17}N_2O_2F_3$	29 (G)	4.16 ± 0.18	25.2 ± 2.9	68%*, 300 μmol/kg
10j[e]	2-pyridyl	98−100	$C_{13}H_{16}N_3O_2F_3$	45 (F)	3.37 ± 0.20	13.0 ± 1.2	68%*, 300 μmol/kg
11a	H	177−179	$C_9H_{15}N_2O_2F_3$	93 (N)	inactive		
11b	Bn	oil	$C_{16}H_{21}N_2O_2F_3$	65 (M)	>1.0		
11c	PhCO	oil	$C_{16}H_{19}N_2O_3F_3$	71 (Q)	0.173 ± 0.027		
11d	$PhSO_2$	oil	$C_{15}H_{19}N_2O_4F_3S$	62 (R)	0.037 ± 0.005	*f*	not active, 30 μmol/kg
11e	4-NC(C_6H_4)CO	oil	$C_{17}H_{18}N_3O_3F_3$	45 (Q)	0.079 ± 0.008	0.25 ± 0.067	67%**, 100 μmol/kg 60%**, 30 μmol/kg 90%, 10 μmol/kg[g]
11f	PhOC(NCN)	60−70	$C_{17}H_{19}N_4O_3F_3$	51 (S)	0.049 ± 0.005	*f*	65%*, 30 μmol/kg
11g	4-PhCO(C_6H_4)CO	88−93	$C_{23}H_{23}N_2O_4F_3$	71 (Q)	0.034 ± 0.006	0.473 ± 0.141	66%*, 30 μmol/kg
11h	CBZ	oil	$C_{17}H_{21}N_2O_4F_3$	51 (N)	0.021 ± 0.002		
11i	*c*HexCO	186−189	$C_{16}H_{25}N_2O_3F_3$	87 (Q)	0.064 ± 0.006	*f*	77%*, 30 μmol/kg
11j	4-NO_2-2-pyridyl	150−152	$C_{14}H_{17}N_4O_4F_3$	21 (J)	0.112 ± 0.011		not active, 30 μmol/kg raises glucose
11k	1-naphthoyl	oil	$C_{20}H_{21}N_2O_3F_3$[h]	47 (Q)	0.070 ± 0.005		not active, 100 μmol/kg
11l	3,4,5-tri-OMe(C_6H_2)CO	oil	$C_{19}H_{25}N_2O_6F_3$[h]	68 (Q)	0.040 ± 0.014	0.159 ± 0.054	60%*, 300 μmol/kg 71%*, 30 μmol/kg
11m	SO_2-1-naphth	oil	$C_{19}H_{21}N_2O_4F_3S$	91 (R)	0.025 ± 0.006	*f*	not active, 30 μmol/kg
11n	2,4,6-tri-Me(C_6H_2)$PhSO_2$	205−207	$C_{18}H_{25}N_2O_4SF_3$	66 (R)	0.039 ± 0.011	*f*	
11o	3,5-di-Cl(C_6H_3)CO	oil	$C_{16}H_{16}N_2O_3F_3Cl_2$	68 (Q)	0.028 ± 0.004	*f*	
11p	4-*t*Bu(C_6H_4)SO_2	188	$C_{19}H_{27}N_2O_4SF_3$	94 (R)	0.036 ± 0.006	*f*	
11q[h]	4-PhCO(C_6H_4)CO	145−146	$C_{25}H_{25}N_2O_4F_3$	72 (B)	inactive		
11r[i]	4-PhCO(C_6H_4)CO	oil	$C_{21}H_{20}N_2O_3Cl_2$[k]	67 (B)	15 ± 2.5		
12a	2-MePip	69−71	$C_{10}H_{16}NO_2F_3$	38 (J)	2.0 ± 0.12		
13a[e]	4-Cl-2-pyridyl	161−162	$C_{14}H_{17}N_3O_2F_3Cl$	29 (J)	2.59 ± 0.22	*f*	
13b[e]	Bn	233−235	$C_{16}H_{22}N_2O_2F_3Cl$	51 (J)	47 ± 4.8		
13c[e]	PhCO	160.5−162	$C_{16}H_{19}N_2O_3F_3$	66 (J)	5.62 ± 0.52		
14a	H	134−137	$C_{10}H_{17}N_2O_2F_3$	95 (N)[l]	inactive		
14b	Bn	126−127	$C_{17}H_{23}N_2O_2F_3$	71 (P, J)	0.082 ± 0.034	*f*	not active, 30 μmol/kg
14c	SO_2-1-naphth	96−100	$C_{20}H_{23}N_2O_4F_3S$	60 (J)	0.0045 ± 0.0010	*f*	not active, 30 μmol/kg
14d	3,5-di-Cl(C_6H_3)CO	183−184	$C_{17}H_{18}N_2O_3F_3Cl_2$	68 (Q)	0.0033 ± 0.0003		
14e	4-NC(C_6H_4)CO	172−175	$C_{18}H_{20}N_3O_3F_3$	72 (Q)	0.016 ± 0.002	0.057 ± 0.013	74%**, 10 μmol/kg 90%**, 3 μmol/kg 87%**, 1 μmol/kg
14f	PhOC(NCN)	72−80	$C_{18}H_{21}N_4O_3F_3$	80 (S)	0.0091 ± 0.0023		
14g	SO_2-1-naphth	96−100	$C_{20}H_{23}N_2O_4F_3S$	61 (J)	0.0045 ± 0.0010	*f*	not active, 30 μmol/kg
14h	3,5-di-Cl(C_6H_3)CO	183−184	$C_{17}H_{18}N_2O_3F_3Cl_2$	68 (Q)	0.0033 ± 0.0003	*f*	
14i	4-MeO_2C(C_6H_4)CO	149−151	$C_{19}H_{23}N_2O_5F_3$	52 (Q)	0.019 ± 0.0075		
14j	4-PhCO(C_6H_4)CO	134−138	$C_{24}H_{25}N_2O_4F_3$	58 (Q)	0.0198 ± 0.0033	*f*	
14k	CBZ	112−114	$C_{18}H_{23}N_2O_4F_3$	53 (Q)	0.0143 ± 0.0028		
14l	*c*HexCO	172−174	$C_{17}H_{27}N_2O_3F_3$	55 (Q)	0.013 ± 0.0013		
15a	4-NC(C_6H_4)CO	116−117	$C_{20}H_{24}N_3O_3F_3$[m]	63 (O)	17 ± 2.9		
16a	Bn	111−112	$C_{17}H_{23}N_2O_2F_3$	60 (J)	inactive		
16b	SO_2-1-naphth	166−167	$C_{20}H_{23}N_2O_4F_3S$	63 (J)	49 ± 3.1		

[a] Analytical results (C, H, N) were within ±0.4% of the theoretical value unless otherwise noted. [b] IC_{50} (μM ± standard error) in primary enzymatic assay of PDHK inhibition (ref 34). [c] EC_{50} (μM ± standard error) in cellular assay of increased oxidation of lactate (ref 60).[d] In vivo study in normal Sprague−Dawley rats (n = 6/group); animals were orally dosed (μmol/kg) after a 24-h fast. Lactate expressed as percent of control, 2 h post-dose. [e] Prepared with racemic 3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid. [f] Increased lactate conversion to CO_2 6−10-fold when dosed at 10× the IC_{50} (ref 61). [g] No lactate lowering was observed after dosing of 3 and 1 μmol/kg of **11e**. [h] Hemihydrate. [i] Methyl ether of **11g**. [j] Dichloroacetyl analogue of **11g**. [k] C: calcd 60.15, found 59.29. [l] Reduction conditions same as the removal of the CBZ moiety in procedure L. [m] Crystals retained 0.25 equiv of hexane; calculated. **$p < 0.05$

5c, compare **11g** with **11q**). However, metabolic activation of these *O*-functionalized derivatives in vivo can afford the active moiety: i.e., **11q** is converted to **11g** upon oral dosing and consequently is effective in activating the PDH complex and lowering lactate as a prodrug.[50]

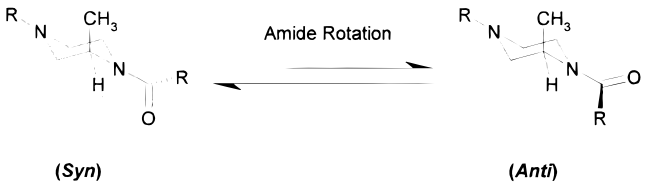


Figure 4. Effects of methyl substitution on the piperazine. The methyl moiety favors the syn disposition due to *A* strain. This *A* strain also destabilizes the ground state, possibly decreasing the energy needed for rotating the amide bond to the binding conformation.

The above data suggested the (*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionyl moiety was the optimal acyl moiety for PDHK inhibition. Thus our attention focused upon the optimization of the amine moiety of the inhibitors.

Considering that **4s**, **5a(*R*)**, **6a(*R*)**, and **7a(*R*)** were approximately equal in potency, it is unlikely that the piperidine ring of **4s** would present the optimal 3-D presentation of the *N*-1 and the *C*-4 substituents. Indeed, the presentation of the 4-substituent of **1** and **4s** must be different due to the different degrees of saturation of the six-membered ring. To obtain an ideal intermediate angle of presentation and to create a more versatile target for SAR exploration, 1,4-disubstituted derivatives of piperazines and homopiperazines were examined. The initial compounds **10c**−**j** and **13a**−**c** also displayed modest but significant activity. Compounds **10i** and **10j** were evaluated for their ability to lower lactate, the most proximal effect of PDHK inhibition in 24-h fasted normal Sprague−Dawley rats, and both presented a modest but significant lowering of lactate 2 h post-oral-dose (300 μmol/kg, see Table 2). Encouraged by these results, a significant effort focused upon improving the potency of the piperazine moiety.

It was postulated that the addition of a methyl substituent on the piperazine ring could increase potency by any of three mechanisms. The first possibility was a beneficial hydrophobic interaction of the methyl moiety with the kinase. The second possibility was a restriction of the preferred ground state of the rotatable phenyl or acyl moieties. For example, it is known that in unsymmetrical amides the bulkier substituent prefers a syn disposition with respect to the carbonyl group (Figure 4).[51,52] The third possibility was, since the methyl group would be axial in piperazines due to significant $A^{(1,3)}$ strain,[53] that the *A* value energy of the methyl moiety could be utilized to lower the energy required for rotating the acyl moiety of the inhibitor to that of its binding conformation.[54] If for instance the optimal conformation of the acyl moiety is a rotation of 10° away from the solution ground state, the 2-methyl moiety will raise the ground state by ∼1.9 kcal/mol. In an analogous situation, it was reported that the barrier of rotation of *N*-acetyl-2-methylpiperidines is lowered by 1.9 kcal/mol as compared to that of *N*-acetylpiperidine, which is attributed to the *A* value of the methyl group.[55,56]

Following this design strategy, monosubstitution of a methyl group in the 3-position or 2-position markedly (≥30-fold) increased potency [i.e., compare **10e** (IC_{50} = 6.7 μM) to **11e** (IC_{50} =70 nM) and **14e** (IC_{50} = 16 nM), compare any of **10b**−**j** to their analogues in **11b**−**o** and **14b**−**l**]. The methyl substitution was superior to others: substitution with larger moieties (i.e., an isopropyl, phenyl, or carboxyl moiety) at these positions afforded inactive or less potent compounds [i.e., compare **14e** (IC_{50} = 16 nM) and **15** (IC_{50} = 17 μM)]. Indeed, most of the increase in potency in the in vitro assay of this series came from this systematic placing of methyl groups on the piperazine ring. In each substitution there was a strong stereochemical preference [3-(*R*) and 2-(*S*), i.e., compare **14b** (IC_{50} = 82 nM) and **14c** (IC_{50} = 4.5 nM) to **16a** (IC_{50} > 300 μM) and **16b** (IC_{50} = 49 μM)].

Of the three possible explanations offered above for the increased potency of the methyl-substituted compounds over the desmethyl derivatives, the role of $A^{(1,3)}$ strain in lowering the energy barrier to obtain the optimal binding conformation of the *N*-1 and *N*-4 substituents is the most plausible. The first explanation, a hydrophobic pocket for the methyl moiety, cannot alone be responsible, since only 0.8 kcal of energy would be expected for optimal binding of a methyl moiety.[57] Even if optimal fit was realized, this potential binding energy is not sufficient for the observed increase in potency. In addition, the 3-methyl substituent increases the potency of the trifluoromethyl-2-hydroxy-2-methylpropionamide derivatives more than that of the dichloroacetamide series. At first glance, one may conclude that the favoring of the syn disposition of the amide group is clearly in evidence due to the strong absolute chirality preference. However, most of the difference is likely due to an unfavorable steric clash in the undesired stereoisomer,[58] as the four stereoisomeric forms of 1,4-dibenzoyl-2,5-*trans*-diethylpiperazine are all significantly populated.[59]

The potent inhibitors described above were evaluated for their ability to increase the conversion of [^{14}C]lactate into $^{14}CO_2$ in human fibroblasts as a measure of their activation of the PDH complex in a modification of Ofenstein's assay (Table 1).[60] Compounds **4i**, **5a**, **10j**, **11e**, **11g**, **11l**, and **14e** had an EC_{50} in the cellular assay of less than 15 μM. The typical magnitude of the maximal increase of lactate conversion to CO_2 was 600−1000% of control. Potency of the compounds in the cellular assay usually correlated well to their potency in the primary enzymatic assay. However, the amount of lactate utilized by cells dropped at higher concentrations of 3-methyl-4-*N*-aryl-substituted piperazines (exemplified by **11j**),[61] suggesting an unfavorable secondary mechanism, not noted with the acyl or sulfonyl derivatives.

The acyl and sulfonyl derivatives were then profiled for their ability to lower lactate, the most proximal effect of PDHK inhibition, in 24-h fasted normal animals.[62] Lactate levels were measured 2 h post-oral-dosing of compounds in 24-h fasted normal Sprague−Dawley rats. A variety of 4-acyl-substituted piperazines were potent inhibitors of PDHK. Of these, **11k** (IC_{50} = 70 nM) and **11l** (IC_{50} = 40 nM) were quite potent and thus were selected for in vivo evaluation. The naphthoyl derivative **11k** did not show any effect. Compound **11l** diminished lactate significantly 2 h post-oral-dose of 300 μmol/kg. The sulfonamide analogues of the acyl compounds, while more potent in the primary in vitro assay and the cellular assay, are not as potent or efficacious at lowering lactate. The sulfonamide **11n**, which possessed the best in vivo profile, had 2,6-dimethyl substitution

Table 3. Pharmacokinetic Parameters upon Oral Dosing of PDHK Inhibitors

pharmacokinetic parameters	**11l** (300 μmol/kg) average ± SD (n = 6)	**11e** (30 μmol/kg) average ± SD (n = 4)
AUC $(\mu M \cdot h)_{0-24}$	127 ± 21.4	196 ± 51.7
T_{max} (h)	1.8	1.25 ± 0.5
C_{max} (μM)	33.7 ± 5.9	23.8 ± 1.42

on the aromatic ring, which may sterically screen the sulfonamide from hydrogen binding and allow better transport across cellular and mitochondrial membranes.

To aid in the design of future analogues and to optimally select further candidates for in vivo studies, a bioavailability study of **11l** was conducted (see Table 3). Although **11l** is orally available, its 3,4,5-trimethoxybenzoyl ring was demonstrated to be oxidized in vivo when dosed orally. From a mass spectrophotometric fragmentation analysis of the metabolites, the only site of metabolism of **11l** observed was on the 3,4,5-trimethoxybenzoyl ring (significantly, no metabolism or derivatization of the 3,3,3-trifluoro-2-hydroxy-2-methylpropionamide moiety was evident). Two major metabolites were detected. One possessed a parent ion of M + 17, which is likely due to aromatic hydroxylation. The other possessed a parent ion of M + 53. It was hypothesized that aroyl groups with an electron-withdrawing substituent (i.e., **11e** and **11g**), although less potent in the in vitro assay, would be less prone to oxidative metabolism and therefore may be superior in overall in vivo properties. In addition, the cyclohexoyl derivative **11i** was selected for in vivo study.

Compounds **11e**, **11g**, and **11i** were equally or more potent and efficacious in vivo than **11l** (see Table 2). Their effect on lowering lactate was also of longer duration (data not shown). Compound **11e** exhibited a higher AUC $(\mu M \cdot h)_{0-24}$ (when orally dosed at a 10-fold lower dose) than **11l** (see Table 3, compound **11g** and **11i** were not profiled).

To confirm that lactate lowering was a consequence of PDHK inhibition and thereby PDH activation, we measured PDH activity ex vivo in treated and untreated control animals. The PDH complex activity was measured in an arylamine acetyltransferase-coupled spectrophotometric assay which was normalized for milliunits of PDH activity/unit of citrate synthase activity for compound- and vehicle-treated animals.[63] The PDH activity was elevated in animals treated with 30 μmol/kg of **11e** or **11g** versus control animals (see Figure 5).

Two lines of reasoning led us to expect that the dimethylated analogues **14c**−**l** would exhibit improved in vivo potency. First, they demonstrated a general 4−8-fold increased potency in our primary screens versus the corresponding methylated analogues **11c**−**o**. Second, since increased lipophilic steric encumbrance near an amide bond can decrease the energy of solvation of an amide, it may be expected that an increased amount of the orally dosed inhibitor may cross the numerous membranes necessary to reach the PDHK target in the physiological setting. The dimethylated analogues were each 5−10-fold more potent than their monomethylated analogues (e.g., compare **14e** and **14g** to **11e** and **11g**). However, no increase in efficacy was discernible, suggesting that these compounds may have reached the limit for PDH activation.

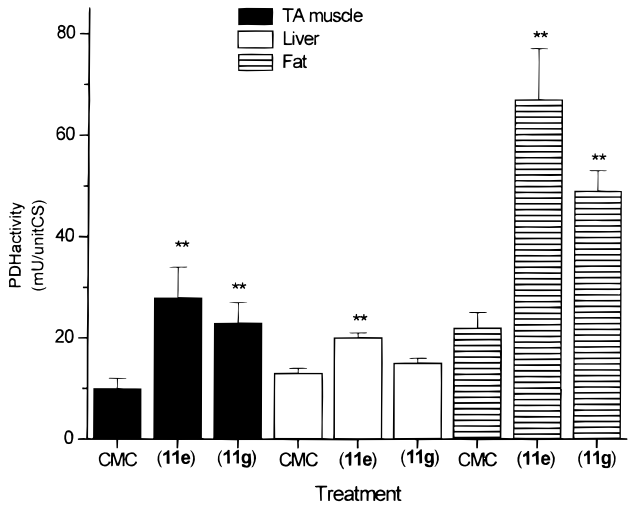


Figure 5. PDH complex activation by **11e** and **11g** in normal Sprague−Dawley rats. Effect of **11e** and **11g** on PDH activity in tibalis anterior muscle, liver, and fat tissues from normal 24-h fasted Sprague−Dawley rats (weight 200 g) 2 h post-oral-dose, 20 μmol/kg po (n = 6/CMC group and **11e** group, n = 3/**11g** group). The PDH complex activity was measured in an arylamine acetyltransferase-coupled spectrophotometric assay and was normalized for citrate synthase activity (see ref 63). $^{**}p < 0.05$ vs the CMC-treated group.

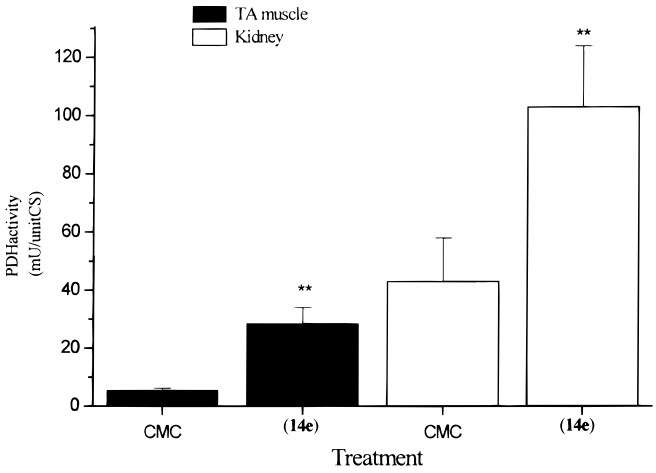


Figure 6. PDH complex activation by **14e** in diabetic Zucker rats. Effect of **14e** on PDH activity in tibalis anterior muscle, liver, and fat tissues from 24-h fasted Zucker diabetic rats (3 μmol/kg/day, 11 days of dosing, 5 h post-oral-dose, n = 8/group). The PDH complex activity was measured in an arylamine acetyltransferase-coupled spectrophotometric assay and was normalized for citrate synthase activity (see ref 64). $^{**}p < 0.05$ vs the CMC-treated group.

Having demonstrated that the PDHK inhibitors **11g** and **14e** are orally active, i.e., lower lactate concentrations in normal animals at low oral doses, we proceeded to investigate their effects in disease models for type 2 diabetes. In diabetic animal models, both **11g** and **14e** elevated PDH activity ex vivo (i.e., see Figure 6). In studies in Zucker diabetic animals and *ob*/*ob* mice, **14e** with a dose of 30 μmol/kg lowered lactate levels (p < 0.05) by greater than 40% in both animal models 2.5 and 5 h post-oral-dosing on day 1 of the study. After day 1 of the study, chronic fed and fasting conditions were studied (data not shown). However, no marked glucose lowering effect was noted upon oral dosing of either of these PDHK inhibitors in either animal model even though the compounds increased PDH complex

activity ex vivo (i.e., see Figure 6). In contrast, DCA treatment effected a significant lowering of blood glucose.

It was recognized early in the program that DCA also affected enzymes other than PDHK.[23,64,65] DCA can affect free fatty acid oxidation both directly (by its affect on short-chain and medium-chain acyltransferases) and indirectly via the PDH complex's effect on carnitine palmitoyltransferase (CPT).[66,67,68] DCA's direct effect on fatty acid oxidation may be relevant in explaining why DCA lowered blood glucose, while **14e** and **11g** did not.

In conclusion, we report the identification of several potent, orally available PDHK inhibitors. For example, (+)-1-*N*-[2,5-(*S*,*R*)-dimethyl-4-*N*-(4-cyanobenzoyl)piperazine]-(*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (**14e**) inhibits PDHK in the primary enzymatic assay with an IC_{50} of 16 ± 2 nM, enhances the oxidation of [^{14}C]lactate into $^{14}CO_2$ in human fibroblasts with an EC_{50} of 57 ± 13 nM, diminishes lactate significantly 2.5 h post-oral-dose at doses as low as 1 μmol/kg, and increases the ex vivo activity of PDH in muscle, liver, and fat tissues. However, these efficacious PDHK inhibitors do not lower blood glucose as was thought to be possible for the treatment of type 2 diabetes. Nevertheless, they are effective at increasing the utilization and disposal of lactate and should be of utility to ameliorate conditions of inappropriate lactate elevation.

Experimental Section

Chemistry. All melting points (mp) were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton magnetic resonance spectra were recorded on a Bruker AC 300-MHz spectrometer. Chemical shifts were recorded in ppm (δ) and are reported relative to the solvent peak or TMS. Mass spectra were run on a Finnigan Mat 4600 spectrometer. Elemental analyses, performed by Robertson Labs, are within 0.4% of theoretical values unless otherwise indicated. Thin-layer chromatography (TLC) was carried out on Macherey-Nagel Polygram Sil G/U_{254} plates. Column chromatography separations were carried out using Merck silica gel 60 (mesh 230−400). Reagents and solvents were purchased from common suppliers and were utilized as received. All reactions were conducted under a nitrogen atmosphere. Yields are of purified product and were not optimized. All starting materials were commercially available unless otherwise indicated.

Method A. *N*′-Methyl-*N*-(4-benzylpiperidine)thiourea (4a). Methyl isothiocyanate (2.00 g, 27 mmol) was added to a solution of 4-benzylpiperidine (4.79 g, 27 mmol) in CH_2Cl_2 at 0 °C. The mixture was warmed to room temperature, stirred for 20 min, and concentrated. The white solid was triturated with hexane to afford **4a** as a white powder (6.54 g, 97%): mp 101−102 °C; ^{1}H NMR ($CDCl_3$) δ 1.29−1.45 (dddd, J = 12.8, 12.5, 11.6, 4.1 Hz, 2H), 1.77 (m, 2H), 1.85 (m, 1H), 2.59 (d, J = 7.0 Hz, 2H), 3.03 (ddd, J = 12.8, 12.8, 1.8 Hz, 2H), 3.19 (s, 3H), 3.27 (bs, 1H), 4.54 (m, 2H), 7.13−7.37 (m, 5H); MS (DCI, NH_3) m/z (rel intensity) 251 (3), 250 (12), 249 (100). Anal. ($C_{14}H_{20}N_2S$) C, H, N.

Method B. 4-Benzylpiperidine, Dichloroacetamide (4i). Dichloroacetyl chloride (3.0 mL, 31.0 mmol) was added to a solution of 4-benzylpiperidine (5.26 g, 30.0 mmol) and triethylamine (4.3 mL, 31 mmol) in CH_2Cl_2 (200 mL) at 10 °C. The mixture was warmed to room temperature, stirred for 1 h, washed with water, 0.05 M HCl, and water, dried (Na_2SO_4), and concentrated. The residue was crystallized from ether and hexanes to afford **4i** as white crystals (5.7 g, 71%): mp 62−63 °C; ^{1}H NMR ($CDCl_3$) δ 1.18−1.39 (m, 2H), 1.75−1.93 (m, 3H), 2.56−2.70 (m, 3H), 3.01−3.12 (m, 1H), 4.15−4.20 (m, 1H), 4.47−4.55 (m, 1H), 6.21 (s, 1H), 7.10−7.33 (m, 5H); MS (DCI, isobutane) m/z (rel intensity) 288 (76), 286 (100). Anal. ($C_{14}H_{17}NOCl_2$) C, H, N.

Method C. *N*-(4-Benzylpiperidine)-2-hydroxy-2-methylpropanamide (4f). *N*-(4-Benzylpiperidine)pyruvamide (3.01 g, 12.3 mmol) was added to a 1.0 M solution of methylmagnesium bromide in Et_2O (24.2 mL, 24.2 mmol) at 0 °C. The mixture was stirred for 20 min and was poured upon saturated aqueous NH_4Cl. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated. The white solid was crystallized from a mixture of hexane and CH_2Cl_2 to afford **4f** (1.73, 54%) as white crystals: mp 177 °C; ^{1}H NMR ($CDCl_3$) δ 1.21 (m, 2H), 1.49 (s, 6H), 1.77 (m, 2H), 1.81 (m, 1H), 2.56 (d, J = 7.0 Hz, 2H), 2.75−2.90 (m, 2H), 4.20−4.44 (m, 2H), 4.79 (bs, 1H), 7.10−7.32 (m, 5H); MS (DCI, NH_3) m/z (rel intensity) 263 (11), 262 (100). Anal. ($C_{16}H_{23}NO_2$) C, H, N.

Method D. *N*-(4-Benzylpiperidine)-2-hydroxypropanamide (4g). Sodium borohydride (500 mg, 13.2 mmol) was added to a solution of *N*-(4-benzylpiperidine)pyruvamide (2.45 g, 10.0 mmol) in absolute ethanol (25 mL) at 0 °C. The mixture was warmed to room temperature, stirred for 30 min, and poured into water. The aqueous layer was extracted twice with Et_2O (50 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to afford **4g** (1.93 g, 78%) as a clear oil: ^{1}H NMR ($CDCl_3$) δ 1.21 (m, 2H), 1.49 (d, J = 7.1 Hz, 3H), 1.75−1.90 (m, 3H), 2.50−2.66 (m, 4H), 2.94 (m, 1H), 3.64 (m, 1H), 4.43 (q, J = 7.1 Hz, 1H), 4.59 (bs, 1H), 7.10−7.32 (m, 5H); MS (DCI, NH_3) m/z (rel intensity) 249 (11), 248 (100). Anal. ($C_{15}H_{21}NO_2$) C, H, N.

Method E. *N*-(4-Benzylpiperidine)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (4s). 1,1-Carbonyldiimidazole (810 mg, 5.0 mmol) was added to a solution of 3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid (790 mg, 5.0 mmol) in CH_2Cl_2 (7 mL). After 15 min, 4-benzylpiperidine (0.88 mL, 5.0 mmol) was added. The mixture was stirred overnight and was then partitioned between 1 N HCl and CH_2Cl_2 (15 mL). The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified via column chromatography with 1% MeOH in CH_2Cl_2 as the eluent. The product was crystallized from hexane to afford **4s** (170 mg, 21%) as white crystals: mp 60−62 °C; ^{1}H NMR ($CDCl_3$) δ 1.18−1.30 (m, 2H), 1.62 (s, 3H), 1.75−1.94 (m, 3H), 2.57 (d, J = 7.1 Hz, 2H), 2,83−2.95 (m, 2H), 4.35−4.54 (m, 2H), 5.51 (s, 1H), 7.16−7.37 (m, 5H); MS (DCI, NH_3) m/z (rel intensity) 317 (13), 316 (100). Anal. ($C_{16}H_{20}NO_2F_3$) C, H, N.

Method F. *N*-Piperidine-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (4v). A solution of (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) (1.042 g, 2.0 mmol) in CH_2Cl_2 (2 mL) was added to a mixture of 3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid (316 mg, 2.0 mmol), piperidine (170 mg, 2.0 mmol), and *N*-methylmorpholine (439 μL, 4.0 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The mixture was warmed to room temperature, stirred an additional 2 h, and then filtered through SiO_2 with 1:1 hexane:ethyl acetate. The eluent was concentrated, and the residue was crystallized from hexane/CH_2Cl_2 to afford **4v** (109 mg, 24%) as white crystals: mp 68−69 °C; ^{1}H NMR ($CDCl_3$) δ 1.56−1.75 (m, 6H), 3.65−3.73 (m, 4H), 5.54 (bs, 1H); MS (DCI, NH_3) m/z (rel intensity) 227 (8), 226 (100). Anal. ($C_9H_{14}NO_2F_3$) C, H, N.

Method G. 1-*N*-(4-*N*-Phenylpiperazine)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (10i). To a mixture of *N*-phenylpiperazine (324 mg, 2 mmol), racemic 3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid (316 mg, 2.0 mmol), and 1-hydroxybenzotriazole hydrate (270 mg, 2.0 mmol) in CH_2Cl_2 in a room-temperature bath was added 1,3-dicyclohexylcarbodiimide (412 mg, 2.0 mmol). The mixture was kept at room temperature for 6 h and then filtered. The filtrate was extracted with 2 N HCl (8 mL). The aqueous layer was adjusted to pH 10 with 2 N NaOH and extracted with ether (15 mL). The organic layer was dried ($MgSO_4$) and concentrated. The residue was filtered through a plug of SiO_2, eluting with ethyl acetate. The eluent was concentrated an triturated with hexane to afford **10i** (174 mg, 29%) as a white powder: mp 97−98 °C; ^{1}H NMR ($CDCl_3$) δ 1.78 (s, 3H), 3.20−3.28 (m,

4H), 3.92−4.00 (m, 4H), 5.13 (bs, 1H), 6.91−6.96 (m, 3H), 7.23−7.30 (m, 2H); MS (DCI, NH_3) *m/z* (rel intensity) 304 (15), 303 (100). Anal. ($C_{14}H_{17}N_2O_2F_3$) C, H, N.

Method H. *N*-(2-Chloroaniline)-2-hydroxy-2-methylbutyramide (5f). Thionyl chloride (3.8 mL, 52 mmol) was added dropwise to a solution of 2-hydroxy-2-methylbutyric acid (5.90 g, 50 mmol) in DMA (50 mL) at −10 °C. After stirring for 30 min, 2-chloroaniline (3.95 mL, 37 mmol) was added and the mixture was stirred for 3 h at room temperature. The mixture was diluted in ether, washed with water, 2 N HCl, water, and saturated $NaHCO_3$, dried, and concentrated. The oil was filtered through SiO_2 with 1:1 hexane/ethyl acetate. The eluent was concentrated to afford **5f** (4.74 g, 55%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.98 (t, J = 7.0 Hz, 3H), 1.56 (s, 3H), 1.88 (m, 1H), 2.03 (m, 1H), 2.32 (bs, 1H), 7.03 (m, 1H), 7.26 (m, 1H), 7.35 (m, 1H), 8.43 (m, 1H), 9.22 (bs, 1H); MS (DCI, NH_3) *m/z* (rel intensity) 230 (31), 229 (12), 228 (100). Anal. ($C_{11}H_{14}NO_2ClF_3$) C, H, N.

Method I. *N*-(Cyclohexylamine)-3,3,3-trifluoro-2-hydroxy-2-phenylpropanamide (6b). A 1.0 M solution of titanium tetrachloride in CH_2Cl_2 (7.0 mL, 7.0 mmol) was added to a solution of cyclohexyl isocyanide (0.73 g, 5.84 mmol) in CH_2Cl_2 at 0 °C. After stirring for 1 h, 2,2,2-trifluoroacetophenone (1.12 g, 6.42 mmol) was added to the mixture. The mixture was stirred an additional 2 h, 1 N HCl (50 mL) was added, and the stirring was continued for 1 h. The organic layer was washed with saturated $NaHCO_3$ and brine, dried, and concentrated. The solid was recrystallized from a mixture of hexane and CH_2Cl_2 to afford **6b** (818 mg, 46%) as white crystals: mp 117−118 °C; ^{1}H NMR ($CDCl_3$) δ 1.02−2.00 (m, 10H), 3.82 (m, 1H), 4.94 (s, 1H), 5.90 (bs, 1H), 7.36−7.43 (m, 3H), 7.60−7.64 (m, 2H); MS (DCI, NH_3) *m/z* (rel intensity) 303 (14), 302 (100). Anal. ($C_{15}H_{18}NO_2F_3$) C, H, N.

Method J. (*S*)-3,3,3-Trifluoro-2-(trimethylsiloxy)-2-methylpropionyl Chloride. 1,3-Bis(trimethylsilyl)urea (4.10 g, 20 mmol) was added to a solution of (*R*)-(+)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid (3.16 g, 20 mmol) in CH_2Cl_2 (40 mL) at room temperature. The mixture was stirred overnight and filtered. The solids were washed with CH_2Cl_2 (2 × 10 mL). A few drops of DMF were added to the combined filtrates; the solution was cooled to 0 °C, followed by the dropwise addition of oxalyl chloride (1.75 mL, 20 mmol) over 10 min. After stirring an additional 1 h at 0 °C, the mixture was warmed to room temperature and stirred for 2 h. The solution of the acid chloride can be stored indefinitely and was assumed to have been prepared in quantitative yield.

General acylation conditions: A 0.83 M solution of (*S*)-3,3,3-trifluoro-2-(trimethylsiloxy)-2-methylpropionyl chloride (1.1 equiv) in CH_2Cl_2 was added to a 1 M solution of the amine (1 equiv) in CH_2Cl_2 and Et_3N (2.5 equiv). The mixture was stirred for 4 h, washed with water, and concentrated. To a 1 M solution of the crude silyl ether in MeOH was added 1 N HCl (10% of the volume of the MeOH). The mixture was stirred for 5 h and then diluted with an equal volume of water. The mixture was concentrated in vacuo to remove MeOH and then extracted three times with CH_2Cl_2 (200 mL, then 2 × 100 mL). The crude products were purified via recrystallization from CH_2Cl_2/hexane or via chromatography through silica gel.

Method K. (*R*)-3-Cyclohexyl-5-methyl-5-trifluoromethyl-2,4-oxazolidinedione (8). 1,1-Carbonyldiimidazole (324 mg, 2.0 mmol) was added to a solution of **6a** (478 mg, 2.0 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred for 72 h and filtered through SiO_2 with 1:1 hexane/ethyl acetate. The eluent was concentrated, and the residue was triturated with hexane to afford **8** (442 mg, 83%) as a white solid: mp 67−69 °C; ^{1}H NMR ($CDCl_3$) δ 1.17−1.43 (m, 3H), 1.60−1.70 (m, 3H), 1.71 (s, 3H), 1.81−1.90 (m, 2H), 2.03−2.14 (m, 2H), 3.92 (m, 1H); MS (DCI, NH_3) *m/z* (rel intensity) 267 (13), 266 (100). Anal. ($C_{11}H_{14}NO_3F_3$) C, H, N.

Method L. (*R*)-3-Cyclohexyl-5-methyl-5-trifluoromethyl-4-oxazolidinone (9). Paraformaldehyde (1.50 g, 50 mmol) was added to a solution of **6a** (1.00 g, 4.18 mmol) in toluene (100 mL) with a catalytic amount of *p*-toluenesulfonic acid monohydrate (25 mg). The mixture was refluxed for 16 h, cooled, and concentrated, and the residue was eluted through SiO_2 with 0.5% MeOH in CH_2Cl_2. The major fraction was concentrated, and the residue was crystallized from a mixture of hexane and CH_2Cl_2 to afford **9** (174 mg, 17%) as colorless crystals: mp 50−52 °C; ^{1}H NMR ($CDCl_3$) δ 1.14 (m, 1H), 1.22−1.44 (m, 4H), 1.59 (s, 3H), 1.76 (m, 1H), 1.82−1.93 (m, 4H), 5.04 (d, J = 3 Hz, 1H), 5.12 (d, J = 3 Hz, 1H); MS (DCI, NH_3) *m/z* (rel intensity) 253 (21), 252 (100). Anal. ($C_{11}H_{16}NO_2F_3$) Calcd: C 52.58, H 6.42, N 5.58. Found: C 53.12, H 6.14, N 5.63.

Method M. (−)-1-*N*-[3-(*R*)-Methyl-4-(3,4,5-trimethoxybenzoyl)piperazine]-(*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (11l). 3,4,5-Trimethoxybenzoyl chloride (180 mg, 0.79 mmol) was added to a solution of 3-(*R*)-methyl-1-benzylpiperazine (150 mg, 0.79 mmol) and triethylamine (180 mg, 0.95 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred overnight, concentrated, and filtered through silica gel with 2% MeOH in CH_2Cl_2 to afford 1-*N*-[2-(*R*)-methyl-4-benzylpiperazine]-3,4,5-trimethoxybenzamide as a clear oil (280 mg, 92%): ^{1}H NMR ($CDCl_3$) δ 1.39 (d, J = 6.1 Hz, 3H), 2.07 (m, 1H), 2.19 (m, 1H), 2.66 (m, 1H), 2.83 (m, 1H), 3.39 (m, 1H), 3.43 (d, J = 12.7 Hz, 1H), 3.54 (d, J = 12.7 Hz, 1H), 3.83 (s, 3H), 3.84 (s, 6H), 6.09 (s, 2H), 7.22−7.35 (m, 5H).

A mixture of the benzamide (270 mg, 0.70 mmol), a catalytic amount of 10% palladium hydroxide on carbon (10 mg), and ethanol (5 mL) was shaken overnight in a Parr shaker under hydrogen (50 psi). The mixture was filtered and the filtrate was concentrated to afford 1-*N*-[2-(*R*)-methylpiperazine]-3,4,5-trimethoxybenzamide as a clear oil (210 mg, 100%): ^{1}H NMR ($CDCl_3$) δ 1.36 (d, J = 6.1 Hz, 3H), 2.43 (bs, 1H), 2.45 (m, 1H), 2.75 (ddd, J = 12.3, 11.9, 3.3 Hz, 1H), 2.86 (m, 1H), 2.95 (dd, 11.9, 3.3 Hz, 1H), 3.03 (m, 1H), 3.19 (m, 1H), 3.85 (s, 3H), 3.86 (s, 6H), 4.40 (b, 2H), 6.58 (s, 2H).

(*S*)-3,3,3-Trifluoro-2-(trimethylsiloxy)-2-methylpropionyl chloride (54 mg, 0.22 mmol) was added to a stirred mixture of 1-*N*-[2-(*R*)-methylpiperazine]-3,4,5-trimethoxybenzamide (43 mg, 0.15 mmol) and polystyrene-supported morpholine[42] (50 mg, 4.3 mmol/g, 0.22 mmol) in CH_2Cl_2 (3 mL). The mixture was shaken for 3 h, and tris(2-aminoethyl)ethylamine polystyrene[42] (65 mg, 1.7 mmol/g, 0.11 mmol) was added. The mixture was shaken overnight and filtered. The resin bed was washed with CH_2Cl_2. The combined filtrates were concentrated, and the residue was dissolved in MeOH (5 mL) and 1 M hydrochloric acid (1 mL). After stirring overnight, the solvents were evaporated to afford **11l** as a crude oil (61 mg, purity 83%)[69] which was purified via column chromatography eluting with 1:1 hexane/ethyl acetate (43 mg, 68%): ^{1}H NMR ($CDCl_3$) δ (all peaks are broad due to interconversion of rotamers) 1.36 (d, J = 6.1 Hz, 3H), 1.73 (s, 3H), 3.07−3.30 (m, 3H), 3.85 (s, 3H), 3.86 (s, 6H), 4.07 (m, 1H), 4.30−4.60 (m, 3H), 6.60 (s, 2H); MS (DCI, NH_3) *m/z* (rel intensity) 436 (18), 435 (100). Anal. ($C_{19}H_{25}N_2O_6F_3 \cdot 0.5H_2O$) C, H, N. Compound **11l** was also prepared in larger quantities from **11a** via method Q.

Method N. (−)-1-*N*-[3-(*R*)-Methyl-4-(carbobenzyloxy)-piperazine]-(*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (11h). $NaHCO_3$ (72 g, 860 mmol) and acetone (160 mL) were added to a mixture of (*R*)-(−)-2-methylpiperazine (20.0 g, 200 mmol) in water (250 mL). At 0−5 °C, a solution of 2-nitrobenzenesulfonyl chloride (53.2 g, 240 mmol) in acetone (80 mL) was added dropwise. The mixture was stirred overnight at ambient temperature. Water (200 mL) was added, and the resulting mixture was concentrated in vacuo to 450 mL and then acidified to pH 3 by the addition of 2 N HCl. The mixture was extracted with CH_2Cl_2 (2 × 150 mL). The organic layers were discarded, and the aqueous layer was made basic to pH 9 with 2 N NaOH. The mixture was extracted with EtOAc (3 × 150 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to afford **6** as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.03 (d, J = 6.1 Hz, 3H), 2.35 (dd, J = 10.1, 11.8 Hz, 1H), 2.64−3.05 (m, 4H), 3.62 (m, 1H), 7.55−7.73 (m, 3H), 7.90−7.96 (m, 1H); MS (DCI, NH_3) *m/z* (rel intensity) 287 (8), 286 (100).

A solution of benzyl chloroformate (33 mL) in acetone (40 mL) was added to a mixture of **6**, acetone (200 mL), water (200

mL), and $NaHCO_3$ (50 g) at 0−5 °C. The mixture was stirred at room temperature overnight. Water (200 mL) was added, and the mixture was concentrated to approximately 400 mL. The mixture was then extracted with EtOAc (2 × 150 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to afford a yellow oil (66.6 g): ^{1}H NMR ($CDCl_3$) δ 1.25 (d, J = 7.0 Hz, 3H), 2.77 (ddd, J = 3.5, 12.3, 12.4 Hz, 1H), 2.97 (dd, J = 3.7, 12.5 Hz, 1H), 3.27 (ddd, J = 3.5, 12.3, 12.4 Hz, 1H), 3.61 (d, J = 12.5 Hz, 1H), 3.80 (dd, J = 1.7, 12.3 Hz, 1H), 4.05 (m, 1H), 4.46 (m, 1H), 5.12 (s, 2H), 7.30−7.40 (m, 5H), 7.60−7.75 (m, 3H), 7.93−7.96 (m, 1H); MS (DCI, NH_3) m/z (rel intensity) 421 (9), 420 (100).

To a solution of the yellow oil in DMF (200 mL) was added thiophenol (18.7 mL, 182 mmol) followed by K_2CO_3 (62.5 g, 452 mmol). The mixture was stirred at room temperature for 24 h and then filtered with the aid of Celite. The solids were washed with CH_2Cl_2 (4 × 200 mL), and the combined filtrates were acidified to pH 2 with 1 N HCl (300 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL), and then the aqueous layer was made basic to pH 10 with 2 N NaOH. The aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to a yellow oil (27.6 g): ^{1}H NMR ($CDCl_3$) δ 1.21 (d, J = 7.0 Hz, 3H), 2.64 (ddd, J = 3.7, 12.1, 12.2 Hz, 1H), 2.73 (dd, J = 1.0, 12.5 Hz, 1H), 2.80−2.95 (m, 2H), 3.02 (ddd, J = 3.3, 12.2, 12.5 Hz, 1H), 3.83 (dd, J = 1.0, 12.5 Hz, 1H), 4.22 (m, 1H), 4.46 (m, 1H), 5.09 (s, 2H), 7.30−7.40 (m, 5H); MS (DCI, NH_3) m/z (rel intensity) 236 (11), 235 (100).

A 0.83 M solution of (*S*)-3,3,3-trifluoro-2-(trimethylsiloxy)-2-methylpropionyl chloride (150 mL, 124.5 mmol) in CH_2Cl_2 was added to a solution of the above oil (27.6 g) in CH_2Cl_2 (200 mL) and Et_3N (43 mL, 310 mmol). The mixture was stirred for 4 h, and then the mixture was washed with water and concentrated to a pale yellow oil. To a solution of the silyl ether in MeOH (200 mL) was added 1 N HCl (10 mL). The mixture was stirred for 5 h, diluted with water (150 mL), concentrated in vacuo to 160 mL, and then extracted with CH_2Cl_2 (1 × 200 mL, then 2 × 100 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to an orange/brown oil. The major product was isolated via a filtration of the residue through SiO_2 with 3:2 hexane/ethyl acetate. Concentration of the major fraction afforded **11h** (38.7 g, 51%) as a clear oil: $[\alpha]_D$ = −20.0 (c = 1.05, MeOH); ^{1}H NMR ($CDCl_3$) δ (all peaks are broad due to interconversion of rotamers) 1.12 (bd, J = 7.0 Hz, 3H), 1.71 (s, 3H), 3.07−3.28 (m, 3H), 4.00 (m, 1H), 4.27−4.48 (m, 3H), 4.79 (s, 1H), 5.16 (s, 2H), 7.33−7.43 (m, 5H); MS (DCI, NH_3) m/z (rel intensity) 376 (14), 375 (100). Anal. ($C_{17}H_{21}N_2O_4F_3$) C, H, N.

***N*-1-[3-(*R*)-Methylpiperazine]-(*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (11a).** A catalytic amount of palladium on carbon support (10% palladium by weight, 200 mg) was added to a solution of **11h** (12.5 g, 33.3 mmol) in absolute EtOH (100 mL) in a Parr shaker bottle (500 mL bottle). The vessel was evacuated and filled with hydrogen three times and finally placed under a hydrogen atmosphere of 42 psi. The mixture was shaken for 3 h, then filtered with the aid of Celite. The reaction was repeated twice under identical conditions, and the combined filtrates were concentrated to afford **11a** (22.3 g, 93%) as a white powder: mp 177−179 °C; ^{1}H NMR (CD_3OD) δ 1.08 (d, J = 6.5 Hz, 3H), 2.30−3.10 (m, 6H), 4.38 (m, 1H); MS (DCI, NH_3) m/z (rel intensity) 242 (9), 241 (100). Anal. ($C_9H_{15}N_2O_2F_3$) C, H, N.

Method O. (+)-1-*N*-[2-(*S*)-Isopropyl-5-(*R*)-methyl-4-*N*-(4-cyanobenzoyl)piperazine]-(*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (15a). Oxalyl chloride (4.5 mL, 50 mmol) was added to a solution of *N*-(9-fluorenylmethoxylcarbonyl)-L-valine (17.0 g, 50 mmol) in CH_2Cl_2 (100 mL) and DMF (0.1 mL) at 0 °C. The mixture was warmed slowly to room temperature for 2 h. The crude acid chloride was added to a solution of *N*-benzyl-L-alanine methyl ester (9.65 g, 50 mmol) in CH_2Cl_2 (100 mL) and Et_3N (11.0 mL, 80 mmol) at 10 °C. The mixture was allowed to warm to room temperature over 1 h. The mixture was washed with water, dried, and concentrated. The residue was subjected to column chromatography eluting with 1% MeOH in CH_2Cl_2 to afford the amide (10.3 g, 40%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 0.91 (d, J = 6.7 Hz, 3H), 0.92 (d, J = 6.7 Hz, 3H), 1.36 (d, J = 7.0, 3H), 2.06 (m, 1H), 3.68 (s, 3H), 3.99 (q, J = 7.0 Hz, 1H), 4.21−4.28 (m, 1H), 4.32−4.47 (m, 2H), 4.56 (dd, J = 9.3, 6.0 Hz, 1H), 4.66−4.72 (m, 2H), 5.52 (d, J = 9.1 Hz, 1H), 7.20−7.46 (m, 9H), 7.62 (d, J = 7.3 Hz, 2H), 7.78 (d, J = 7.3 Hz, 2H).

A mixture of *N*-α-(9-fluorenylmethoxycarbonyl)-L-valinyl-*N*-β-benzyl-L-alanine methyl ester (10.3 g, 20.0 mmol) in MeOH (400 mL) and concentrated aqueous ammonia (75 mL) was refluxed overnight and then concentrated. The residue was purified via flash chromatography eluting with 2% MeOH in CH_2Cl_2 to afford the diketopiperazine (3.8 g, 73%) as a white foam. An analytical sample was crystallized from ether and hexanes: mp 88−89 °C; $[\alpha]_D$ = −20.2 (c = 1.0, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.90 (d, J = 6.7 Hz, 3H), 1.06 (d, J = 6.7 Hz, 3H), 1.47 (d, J = 7.0 Hz, 3H), 2.69 (m, 1H), 3.85 (quartet, J = 7.0 Hz, 1H), 3.96 (d, J = 7.0 Hz), 3.97 (d, J = 14.9 Hz, 1H), 5.36 (d, J = 14.9 Hz, 1H), 6.19 (bs, 1H), 7.18−7.40 (m, 5H).

A solution of the diketopiperazine (3.00 g, 11.5 mmol) in THF (25 mL) was added dropwise to a refluxing mixture of $LiAlH_4$ (2.0 g, 52 mmol) in THF (50 mL). The mixture was refluxed for 3 h, then stirred overnight at room temperature. The mixture was quenched with saturated $MgSO_4$ solution, and the aluminate salts were filtered. The salts were extracted several times with ether. The combined filtrates were combined and concentrated to the crude piperazine as an oil (2.5 g, 94%): ^{1}H NMR ($CDCl_3$) δ 0.87 (d, J = 6.7 Hz, 3H), 0.92 (d, J = 6.7 Hz, 3H), 1.16 (d, J = 6.1 Hz, 3H), 1.89 (t, J = 11.0 Hz, 1H), 2.25−2.45 (m, 2H), 2.66 (m, 1H), 2.81 (dd, J = 11.9, 2.6 Hz, 1H), 3.08 (dd, J = 11.9, 2.8 Hz, 1H), 3.17 (d, J = 13.5 Hz, 1H), 4.10 (d, J = 13.5 Hz), 7.24−7.40 (m, 5H).

The crude oil was acylated via method J to afford the benzyl amine: mp 116−117 °C; $[\alpha]_D$ = 46.8 (c = 1.00, MeOH); ^{1}H NMR δ 0.75 (d, J = 6.4 Hz, 3H), 0.76 (d, J = 6.7 Hz, 3H), 0.99 (d, J = 6.7 Hz, 3H), 1.66 (s, 3H), 2.47−2.68 (m, 3H), 3.06 (m, 1H), 3.44 (d, J = 13.2 Hz, 1H), 3.52 (m, 1H), 3.64 (d, J = 13.2 Hz), 3.87 (m, 1H), 4.25 (m, 1H), 5.28 (bs, 1H), 7.22−7.38 (m, 5H). Anal. ($C_{19}H_{27}N_2O_2F_3$) Calcd: C 61.28, H 7.31, N 7.52. Found: C 61.46, H 7.52, N 7.45.

This benzyl amine was reduced via method N to afford the amine: mp 136−137 °C; $[\alpha]_D$ = 16.0 (c = 1.00, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.79 (d, J = 6.6 Hz, 3H), 0.99 (d, J = 6.6 Hz, 3H), 1.21 (d, J = 7.0 Hz, 3H), 1.65 (s, 3H), 2.40 (m, 1H), 2.92 (d, J = 12.9 Hz, 1H), 3.15 (dd, J = 13.6, 4.0 Hz, 1H), 3.24 (m, 1H), 3.44 (m, 1H), 3.94 (d, J = 13.3 Hz, 1H), 4.27 (m, 1H); yield 2.70 g, 63%.

Acylation of the amine with 4-cyanobenzoyl chloride via method Q afforded **15a** as white crystals: mp 116−117 °C; $[\alpha]_D$ = 46.8 (c = 1.0, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.60−1.70 (m, 9H), 2.02 (m, 1H), 2.82−5.04 (m, 7H), 7.43 (m, 2H), 7.71 (d, J = 8.4 Hz, 2H); MS (DCI, NH_3) m/z (rel intensity) 413 (13), 412 (100). Anal. ($C_{20}H_{24}N_3O_3F_3$) C, H, N, F.

Method P. Resolution of *trans*-2,5-Dimethyl-4-benzylpiperazine. To a solution of racemic *trans*-2,5-dimethyl-4-benzylpiperazine (59 g, 0.29 mol) in MeOH (150 mL) was added a solution of (−)-tartaric acid (87 g, 0.58 mol) in MeOH (250 mL) dropwise for 5 min. Crystallization began upon addition, and the solution was stored at 0 °C overnight. The mixture was filtered, washed with cold MeOH (100 mL), and dried to afford the ditartaric acid salt of (*R*,*S*)-2,5-dimethyl-4-benzylpiperazine (73.9 g) as white crystals: mp 103−104 °C; $[\alpha]_D$ = −41.0 (c = 1.00, MeOH).

A single recrystallization from MeOH (cooling to room temperature) afforded the salt with >98% ee as white crystals (58.0 g, 79%): mp 118.5−120 °C; $[\alpha]_D$ = −45.7 (c = 1.00, H_2O); ^{1}H NMR (D_2O) δ 1.18 (d, J = 6.5 Hz, 3H), 1.51 (d, J = 6.3 Hz, 3H), 1.92 (bs, 1H), 2.89 (t, J = 12.9 Hz, 1H), 3.18 (t, J = 13.8 Hz, 1H), 3.29 (dd, J = 13.8, 3.0 Hz, 1H), 3.47 (m, 1H), 3.53 (m, 1H), 3.62 (dd, J = 12.9, 3.0 Hz, 1H), 3.98 (d, J = 13.1 Hz, 1H), 4.41 (s, 4H), 4.71 (d, J = 13.1 Hz, 1H), 7.39−7.48 (m, 5H); MS (DCI, NH_3) m/z (rel intensity) 206 (14), 205 (100). Anal. ($C_{21}H_{32}N_2O_{12}$) C, H, N.

Method Q. 1-*N*-[4-*N*-(4-Cyanobenzoyl)piperazine]-(*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (10e). 4-Cyanobenzoyl chloride (448 mg, 2.7 mmol) was added to a suspension of **10a** (560 mg, 2.48 mmol) in CH_2Cl_2 (10 mL) and Et_3N (320 mg, 3.16 mmol) at 15 °C. The mixture was stirred for 1 h, washed with water and 0.5 N HCl, dried, and concentrated. The residue was crystallized from ether to afford **10e** (570 mg, 65%) as white crystals: mp 173−174 °C; $[\alpha]_D = -2.0$ ($c = 0.83$, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.69 (s, 3H), 3.35−3.90 (broad peaks, 8H), 4.63 (s, 1H), 7.50 (d, $J = 7.9$ Hz, 2H), 7.73 (d, $J = 7.9$ Hz, 2H); MS (DCI, NH_3) *m*/*z* (rel intensity) 375 (25), 356 (19), 166 (89), 143 (100). Anal. ($C_{16}H_{16}N_3O_3F_3$) C, H, N, F.

Method R. (−)-1-*N*-[3-(*R*)-Methyl-4-*N*-(4-*tert*-butylbenzenesulfonyl)piperazine]-(*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (11p). To a solution of 4-*tert*-butylbenzenesulfonyl chloride (116 mg, 0.50 mmol) in CH_2Cl_2 (3 mL) was added *N*-1-[2-(*R*)-methylpiperazine]-(*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (120 mg, 0.50 mmol) followed by Et_3N (100 μL). The mixture was set aside overnight and filtered through SiO_2 with 1:1 hexane:ethyl acetate. The eluent was concentrated to a paste. The residue was triturated with a mixture of hexanes and ether and filtered to afford **11p** (198 mg, 94%) as white crystals: mp 188 °C; $[\alpha]_D = -10.6$ ($c = 1.00$, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.02 (d, $J = 7.0$ Hz, 3H), 1.33 (s, 9H), 1.66 (s, 3H), 3.02−3.22 (m, 3H), 3.63−3.72 (m, 1H), 4.15−4.37 (m, 3H), 4.45 (s, 1H), 7.50 (d, $J = 8.4$ Hz, 2H), 7.71 (d, $J = 8.4$ Hz, 2H). Anal. ($C_{19}H_{27}N_2O_4SF_3$) C, H, N.

Method S. (−)-1-*N*-[4-*N*-((*R*)-3,3,3-Trifluoro-2-hydroxy-2-methylpropionyl)-(*R*,*S*)-2,5-dimethylpiperazine]carboximidic Acid, *N*-Cyanophenyl Ester (14f). A solution of *N*-1-[2,5-(*S*,*R*)-dimethylpiperazine]-(*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (80 mg, 0.32 mmol) and diphenyl cyanocarbonimidate (78 mg, 0.32 mmol) in THF (2 mL) was heated for 16 h at 60 °C. The mixture was concentrated and the residue was eluted through SiO_2 with 2% MeOH in CH_2Cl_2. The major fraction was concentrated to afford **14f** (100 mg, 80%) as a white solid: mp 72−80 °C; $[\alpha]_D = -31.4$ ($c = 0.70$, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.20−1.35 (b, 6H), 1.67 (bs, 3H), 3.15−5.00 (m, 7H), 7.07 (d, $J = 7.8$ Hz, 2H), 7.29 (m, 1H), 7.41 (m, 2H); MS (DCI, NH_3) *m*/*z* (rel intensity) 400 (20), 399 (100). Anal. ($C_{18}H_{21}N_4O_3F_3$) C, H, N.

Biological Procedures. (1) PDHK Primary Enzymatic Assay. The primary enzymatic assay was performed using commercially available PDH complex as described previously by spectrophotometrically measuring the production of NADH at 340 nM in the presence and absence of ATP.[34,35,70]

(2) Cellular PDH Assay. The cellular PDH assay was performed as described previously[60] with the exception that PDH activity was assayed in cells in suspension rather than in confluent cells. Cells were harvested using trypsin 2−4 days after reaching confluence. Aliquots of cells (200 μL, $(3.5-5) \times 10^5$ cells) were transferred to plastic tubes containing 25 μL of test compound solution or solvent control. The tubes were equilibrated to 37 °C and the PDH assay was initiated by the addition of 25 μL of DMEM-S medium containing 25 μM lactate and 0.2 μCi/mL d,l-[1-^{14}C]lactate. Each tube was briefly gassed (95% O_2, 5% CO_2) and a plastic collection cup was introduced into each tube, suspended from a rubber septum used to seal the neck of the tube. The tubes were slowly shaken for 25 min at 37 °C. The assay was terminated by addition of 250 μL of 12% w/v trichloroacetic acid. The tubes were chilled on a block of ice and 100 μL of Solvable (NEN Research Products, Boston, MA) was added to the plastic collection cups in order to collect released $^{14}CO_2$. After a 30-min incubation collection cups containing Solvable were transferred to vials containing scintillation fluid, and radioactivity was determined by liquid scintillation counting.

(3) Measurement of Lactate Lowering in Normal Animals. Normal male Sprague−Dawley rats weighing approximately 200 g were randomly assigned to vehicle- or compound-treated groups. After a 24-h fast the animals were dosed with either vehicle (0.5% carboxymethylcellulose with 0.2% Tween-80, 1 mL/100 g of body weight) or compound in vehicle. Blood samples were taken 2 h after the dosing and were collected in microcentrifuge tubes containing heparin and fluoride to prevent blood clotting and inhibit glycolysis. Blood lactate and glucose concentrations were determined using a YSI 2700 dual channel biochemistry analyzer (Yellow Springs Instrument Co., Yellow Springs, OH). A two-tailed and unpaired *t*-test was used to compare the differences between the two groups.

(4) Evaluation of Pharmacokinetic Parameters. Normal Sprague−Dawley rats were dosed as above. Two additional rats provided plasma for a calibration curve. Blood samples were taken prior to dosing (0 h) and 0.25, 0.5, 1, 2, 4, 8, and 24 h post-oral-dosing. Samples collected in heparinized tubes were centrifuged, and the concentration of compound in the supernatant was determined by LC/MS.

(5) Arylamino Acetyltransferase-Coupled Spectrophotometric ex Vivo Assay. Pyruvate dehydrogenase activity in tissue extracts was determined spectophotometrically according to a modification of described methods[63] allowing for microtiter plate measurement as follows: For each assay, a 10-μL portion of tissue extract [diluted in 0.1 M tris(hydroxymethyl)aminomethane-HCl, pH 7.8] was added to a well of a 96-well microtiter plate. To this was added a 170-μL portion of assay buffer [100 mM tris(hydroxymethyl)aminomethane-HCl, pH 7.8, 0.5 mM EDTA, 1 mM magnesium chloride, 50 mM sodium fluoride, 500 μM nicotinamide adenine dinucleotide, 1 mM thiamine pyrophosphate, 100 μM coenzyme A, 20 μg/mL *p*-(*p*-aminophenylazo)benzenesulfonic acid] containing arylamine acetyltransferase. The mixture was incubated for 10 min in a Molecular Devices Thermomax or Spectromax 250 spectrophotometer set at 30 °C in order to deplete any acetyl-CoA present in the tissue extract. The PDH assay was initiated by the addition of 1 mM pyruvate (20 μL/well), and conversion of *p*-(*p*-aminophenylazo)benzenesulfonic acid to acetyl-*p*-(*p*-aminophenylazo)benzenesulfonic acid was monitored at 450 nm (kinetic mode) for 30 min. The PDH complex activity was normalized for citrate activity. Citrate synthase activity was determined essentially according to Srere et al.[72] For each assay, a 10-μL portion of tissue extract [diluted in 0.1 M tris(hydroxymethyl)aminomethane-HCl, pH 7.8] was added to 190 μL of a buffer containing 100 mM tris(hydroxymethyl)aminomethane-HCl (pH 7.4), 50 μM acetyl-CoA, 100 μM oxaloacetate, and 100 μM dithiobis(2-nitrobenzoic acid). The production of mercaptide ion was monitored at 405 nm (kinetic mode) for 10 min using a Molecular Devices Thermomax or Spectromax 250 spectrophotometer set at 30 °C.

(6) Evaluation of Compounds in Diabetic Animal Models.[73] Fed adult male C57BL *ob*/*ob* mice (Jackson Labs, Bar Harbor, ME) were distributed in groups of 10 and matched for blood glucose on day 0. Food was withheld for 4.5 h prior to dosing with either vehicle (0.5% carboxymethylcellulose with 0.2% Tween-80, 1 mL/100 g of body weight) or compound in vehicle. Blood samples were taken 1, 2.5, and 5 h after the dosing and were collected in microcentrifuge tubes containing heparin and fluoride to prevent blood clotting and inhibit glycolysis. Blood lactate and glucose concentrations were determined using a YSI 2700 dual channel biochemistry analyzer (Yellow Springs Instrument Co., Yellow Springs, OH). A two-tailed and unpaired *t*-test was used to compare the differences between the two groups.

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(69) This crude oil was of sufficient purity to be tested in the primary enzymatic assay. The resulting IC_{50}'s of crude substances made this way were in acceptable agreement (within 25%; i.e., 40 nM vs 50 nM) with the values of the purified substances. All IC_{50}'s reported within this paper are of purified substances.

(70) A reviewer suggested that the compounds may directly activate the PDH complex. The compounds do not directly activate PDH: i.e, they do not increase the production of NADH from PDH_{active} (see Figure 1). In addition, in a direct assessment of PDHK activity in the PDC preparation via measuring the initial rate of phosphorylation of the E1 subunit of the complex, the IC_{50} for **14e** remains as it is in the primary enzymatic assay (see ref 35 for the details of this assay).

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