(*R*)-3,3,3-Trifluoro-2-hydroxy-2-methylpropionamides Are Orally Active Inhibitors of Pyruvate Dehydrogenase Kinase

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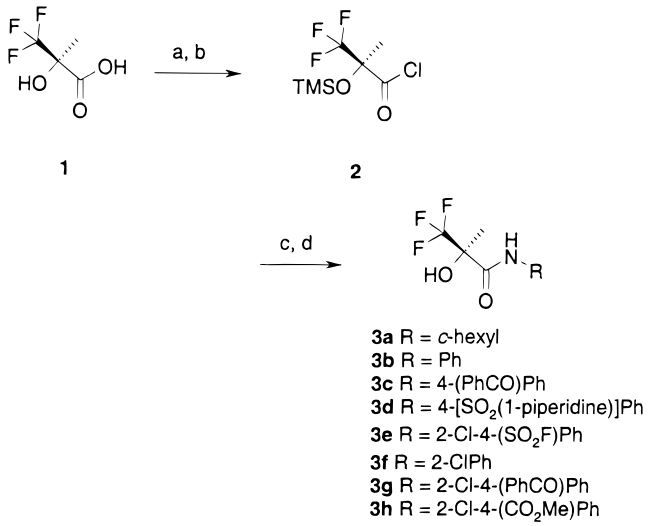
The activity of the pyruvate dehydrogenase (PDH) complex is lower during conditions of reduced oxidative glucose metabolism such as obesity, starvation, and diabetes and in patients with congenital lactic acidosis.[1−6] The PDH complex catalyzes the decarboxylation of pyruvate to acetyl-CoA.[7] The activity 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 and consequently to reduced oxidative glucose metabolism. 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 increases the activity of PDHK. It has been proposed that the reversible acetylation of the lipoamide of the E2 subunit within 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 oxidation of free fatty acids and the oxidation of glucose are related in a reciprocal manner.[13]

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, 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] No compounds other than α,α-dihalogenated carbonyl compounds are known to inhibit PDHK.[30] Herein is reported a program which resulted in structurally novel, orally active inhibitors of PDHK.

Scheme 1. Preparation of Amides[a]



[a] Conditions: (a) 1,3-bis(trimethylsilyl)urea, CH_2Cl_2; (b) $(COCl)_2$, cat. DMF, CH_2Cl_2; (c) Et_3N, CH_2Cl_2, amine; (d) aq HCl, MeOH.

We found that the amide **3b** inhibited PDHK in the primary enzymatic assay.[31] 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.[32] Therefore, systematic exploration of the structural features necessary for more potent inhibitors was initiated.

The synthetic methods used in the preparation of these PDHK inhibitors are shown in Schemes 1−3. (*R*)-3,3,3-Trifluoro-2-hydroxy-2-methylpropionic acid (**1**) can be effectively produced via the enzymatic resolution of the butyrate ester of the racemate of **1**.[33,34] The amides can be made directly from (*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid (**1**) via conversion of the acid to (*S*)-3,3,3-trifluoro-2-(trimethylsiloxy)-2-methylpropionyl chloride (**2**) utilizing a modification of Kelly's procedure (see Scheme 1).[35] In short, the acyl halide was prepared by treating the carboxylic acid **1** with bis-(trimethylsilyl)urea in CH_2Cl_2. After filtration to remove the urea byproduct, the bissilylated acid is converted to the acyl halide **2** by treatment with oxalyl chloride

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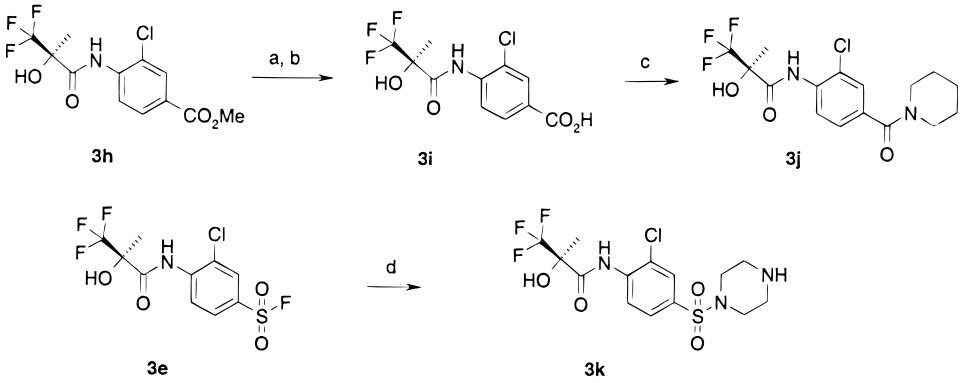
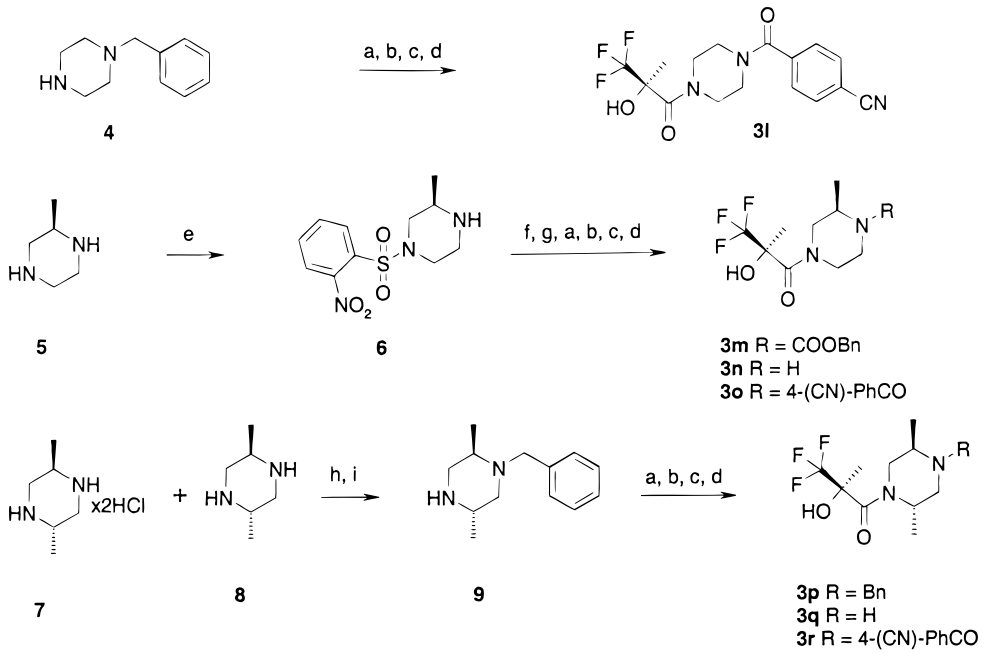
Scheme 2. Elaboration of Anilides **3e**,**h**[a]



[a] Conditions: (a) KOH, EtOH; (b) aq HCl; (c) PyBOP, CH_2Cl_2, piperidine; (d) piperazine, CH_2Cl_2.

Scheme 3. Synthesis of Bisacylated Piperazines[a]



[a] Conditions: (a) Et_3N, CH_2Cl_2, **2**; (b) aq HCl, MeOH; (c) Pd/C, H_2, EtOH; (d) Et_3N, CH_2Cl_2, 4-CN-C_6H_4COCl; (e) 2-(NO_2)-$C_6H_4SO_2Cl$, K_2CO_3, aq acetone; (f) CBZCl, K_2CO_3, aq acetone; (g) C_6H_5SH, K_2CO_3, DMF; (h) EtOH, BnCl; (i) 2 equiv of (−)-tartaric acid, H_2O, recrystallize.

in the presence of a catalytic amount of DMF. The acid chloride **2** can be distilled from the reaction mixture, if desired. However, **2** can also be stored for weeks at room temperature as the crude reaction mixture and utilized as such with no discernible detrimental effect on the coupling yields. The crude α-siloxyamides produced by coupling with an amine are effectively desilylated by methanolic HCl to afford **3a**−**h**,**m**,**p**.

The anilides **3i**−**k** were made via elaboration of the anilides **3e**,**h** (see Scheme 2). Hydrolysis of the methyl ester **3h** with ethanolic KOH afforded **3i**, which upon treatment with the coupling reagent (benzotriazol-1-yloxy)tripyrrolodinophosphonium hexafluorophosphate (PyBOP) and piperidine formed **3j**. Displacement of the fluoride of **3e** with piperazine afforded **3k**.

The obvious route to **3n** was to monoacylate (*R*)-2-methylpiperazine (**5**) with **2** (see Scheme 3). However, monoacetylation of piperazines is difficult,[36] and varied attempts to monoacetylate piperazines with **2** failed. An efficient route to **3n** was to take advantage of the report that 2-substituted piperazines can be monosulfonylated.[37] Employing the protecting moiety developed by Fukayama et al.,[38] (*R*)-2-methylpiperazine (**5**) was cleanly monosulfonylated with 2-nitrobenzenesulfonyl chloride to afford **6**. Consequently, large quantities of **3m** from **5** were obtainable in excellent yield. Reductive cleavage of the carbobenzyloxy moiety gave **3n**, and benzoylation with 4-cyanobenzoyl chloride afforded **3o**.

Optically pure **3r** was conveniently prepared from achiral *trans*-2,5-dimethylpiperazine in six steps by monobenzylation of a 1:1 mixture of its bishydrochloride salt **7** and its free base **8** in absolute ethanol in a modification of Craig and Young's procedure to produce racemic **9**.[39] A single crystallization of the bistartrate salt of **9** from methanol afforded **9** with 90% enantiomeric excess with a second recrystallization affording optically pure **9** in 79% overall theoretical yield. Acylation of the salt of **9** with **2**, followed by reduction over Pd in ethanolic HCl, formed **3q** (the stereochemistry of **3q** was confirmed through an X-ray crystal structure of its HCl salt). Acylation of **3q** with 4-cyanobenzoyl chloride gave **3r**.

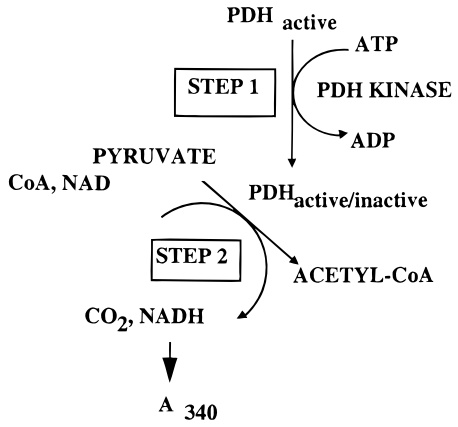


Figure 1. Depiction of PDHK primary assay. Inhibitors of PDHK do not allow the complex to become inactivated in the presence of ATP during step 2. The concentration at which 50% of the original NADH production is maintained is the apparent IC_{50} (see ref 31).

The compounds prepared above were tested in the primary high-throughput assay, which utilizes the commercially available porcine PDH complex[40] that contains intrinsic PDHK activity as reported.[31] Briefly, the assay consists of two experimental steps: In the first step, PDHK catalyzes the ATP-dependent phosphorylation of the PDH complex in the presence or absence of inhibitor. The second step determines the extent of the PDH complex inactivation and is measured spectrophotometrically via the absorbance of the NADH produced by the complex (see Figure 1).

As anticipated, it was found that all of the PDHK inhibitors identified from our high-throughput screening are remarkably selective versus other eukaryotic Ser/Thr/Tyr protein kinases. No compound in this report, nor in our other series of PDH kinase inhibitors, has displayed any significant inhibition of cAMPk or p38 MAP kinase at concentrations below 100 μM as measured by the assays described in the literature.[41,42] The anticipated selectivity was due to the fact that while PDHK isozymes share significant sequence similarity with members of the prokaryotic histidine protein kinases,[43,44] PDHK isozymes share little similarity with the catalytic domain of the eukaryotic Ser/Thr/Tyr protein kinase family.[45]

The compounds' IC_{50}'s for the inhibition of the inactivation of the PDH complex by PDHK are reported in Table 1. Diverse amides of (*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid of structure **3a**,**b**,**l** are modest to potent inhibitors of PDHK. Substitution of the anilide in the *ortho* position with a small electron-withdrawing group increased the potency of the anilide series markedly (i.e., compare **3b**,**c** to **3f**,**g**). Substitution in the *para* position increased the potency of the series modestly (compare **3b** to **3c**,**d**). Although **3g**−**k** and other 4-substituted 2-chloroanilides not reported here were nearly equivalent in their ability to inhibit PDHKs' ability to inactivate the complex, their oral activity in vivo varied.[46] Mono- and disubstitution of the piperazine **3l** in the 3- and 2,5-positions with a methyl moiety markedly increased the potency of the series (compare **3l** to **3p**,**s**). The 4-cyanobenzoyl moiety of **3o**,**r** can be replaced with a wide variety of substituents (acyl, alkyl, aryl, sulfonyl, etc.).[47]

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).[48] Compounds **3c**,**f**,**i**−**k**,**o**,**r** had EC_{50}'s in the cellular assay of less than 20 μM. The typical magnitude of the increase of lactate conversion to CO_2 was 600−1000% of control. Potency of compounds in the cellular assay usually correlated well to their potency in the primary enzymatic assay (e.g., **3o** to **3r**,**b**, **3f** to **3i**). However, the anilides tend to be less potent in the cellular assay than the piperazine derivatives (e.g., compare **3i**,**k** to **3o**).

Excellent oral bioavailability of the anilides and piperazine derivatives was demonstrated upon oral administration of **3i**,**k**,**o** at doses of 30, 100, and 300 μmol/kg. Peak blood plasma concentrations of greater than 20 μM were obtained with each of the compounds (Table 2). Each also had an estimated half-life of at least 3 h. These orally bioavailable compounds were profiled for their ability to lower lactate, the most proximal effect of PDHK inhibition, in 24-h fasted normal animals.[49] Each of these compounds significantly lowered lactate at the 2-h time point (Table 1). As would be expected from the cellular data, the piperazine analogues were more potent than the anilide analogues. The maximal lactate lowering of these compounds was in general statistically equivalent to the maximal effect of DCA. The piperazine **3r**, the most potent compound in vivo described within this report, lowered blood lactate significantly when dosed at 1 μmol/kg in normal fasted rats.

As noted earlier, anilides of (*S*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid have been reported to activate the K_{ATP} channel and are being investigated clinically as potential therapy for urinary incontinence.[32] However, the SAR for each of these targets is very different. Importantly, the (*S*)-enantiomer of the acid is the preferred enantiomer for the K_{ATP} channel opening, while the (*R*)-enantiomer is preferred for PDHK inhibition [compare **3a**,**f** to **3a(*S*)**,**f(*S*)**]. In addition, substitution at the 2-position of an anilide with a halogen has been reported to diminish the potency of K_{ATP} channel openers,[32] while this substitution markedly increases the potency of the PDHK inhibitors. In general, the (*R*)-antipodes of the (*S*)-anilides reported as K_{ATP} inhibitors are modest inhibitors of PDHK (IC_{50}'s of 0.5−20 μM) and can be increased in potency by ∼5−40-fold by 2-substitution of a halogen or a small electron-withdrawing substituent (i.e., Cl, Br, F, or acetyl; compare **3c** to **3g**). **3g**,**i** present no measurable effect on K_{ATP} activity in vitro (IC_{50} > 100 μM) or ex vivo (ED_{50} > 300 μM) utilizing assays described in the literature (data not shown). The few secondary amides of (*S*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid previously described were reported to be devoid of K_{ATP} channel-opening activity.[32] The piperazine analogues reported above similarly have no effect on K_{ATP} channels (data not shown).

In summary, diverse amides of (*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid are the first inhibitors of PDHK reported without halogens α,α to a carbonyl.

Table 1. Physical Characteristics and in Vitro and in Vivo Data of **3a**−**r**

entry	mp (°C)	empirical formula	anal.[a]	% yield	IC_{50} (μM)[b]	EC_{50} (μM)[c]	lactate[d] (% of control)
DCA[e]					>1000	130 ± 60	70% (1 mmol/kg)
3a	79−80	$C_{10}H_{16}NO_2F_3$	C, H, N	73	6.2 ± 0.5		
3a(*S*)[f]	83−84	$C_{10}H_{16}NO_2F_3$	C, H, N	58	200 ± 24		
3b	116	$C_{10}H_{10}NO_2F_3$	C, H, N, F	27	35 ± 1.4		
3c	153	$C_{17}H_{14}NO_3F_3$	lit.[h]	68	9.3 ± 0.8	8.3 ± 0.34	not active (300 μmol/kg)
3d	166	$C_{15}H_{19}N_2O_4F_3S$	C, H, N, F, S	60	9.3 ± 0.4		
3e	137−139	$C_{10}H_8NSO_4F_4Cl$	C, H, N, Cl, S	95	0.077 ± 0.010		
3f	112−114	$C_{10}H_9NO_2ClF_3$	C, H, N, F, Cl	43	0.300 ± 0.028	5.4 ± 0.39	not active (300 μmol/kg)
3f(*S*)[f]	113−114	$C_{10}H_9NO_2ClF_3$	C, H, N, F, Cl	64	9.8 ± 0.57		
3g	140−142	$C_{17}H_{13}NO_3ClF_3$	C, H, N	35	0.110 ± 0.015		
3h	119−121	$C_{12}H_{11}NO_4ClF_3$	C, H, N, F, Cl	68	0.120 ± 0.053		
3i	205−207	$C_{11}H_9NO_4F_3Cl$	C, H, N, Cl	89	0.090 ± 0.015	1.4 ± 0.10	70%** (100 μmol/kg) 98% (30 μmol/kg)
3j	185−186	$C_{16}H_{18}N_2O_3ClF_3$	C, H, N, F, Cl	85	1.50 ± 0.12	0.59 ± 0.17	not active (300 μmol/kg)
3k	227−228	$C_{14}H_{17}N_3SO_4ClF_3$	C, H, N, Cl, F, S	58	0.055 ± 0.0021	17 ± 3.8	72%** (300 μmol/kg)
3l	173−174	$C_{16}H_{16}N_3O_3F_3$	C, H, N	65	6.7 ± 0.97		
3m	oil	$C_{17}H_{21}N_2O_4F_3$	C, H, N	51	0.021 ± 0.002		
3n	177−179	$C_9H_{15}N_2O_2F_3$	C, H, N	93	inactive		
3o	oil	$C_{17}H_{18}N_3O_3F_3$	C, H, N	45	0.079 ± 0.008	0.25 ± 0.067	67%** (100 μmol/kg) 60%** (30 μmol/kg) 90% (10 μmol/kg)[g]
3p	126−127	$C_{17}H_{23}N_2O_2F_3$	C, H, N	71	0.082 ± 0.034		
3q	134−137	$C_{10}H_{17}N_2O_2F_3$	C, H, N	91	inactive		
3r	172−175	$C_{18}H_{20}N_3O_3F_3$	C, H, N	72	0.0165 ± 0.0021	0.057 ± 0.013	74%** (10 μmol/kg) 90%** (3 μmol/kg) 87%** (1 μmol/kg)

[a] Analytical results were within ±0.4% of the theoretical value. [b] IC_{50} (μM ± standard error) in primary enzymatic assay of PDH kinase inhibition (ref 31). [c] EC_{50} (μM ± standard error) in cellular assay of increased oxidation of lactate (ref 48). [d] In vivo study in normal Sprague−Dawley rats (n = 6/group); animals were orally dosed (μmol/kg) after a 24-h fast. Lactate is expressed as percent of control, 2-h postdose. [e] Sodium dichloroacetate. [f] Synthesized from (*S*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid of 96% ee. [g] No lactate lowering was observed after dosing of 3 and 1 μmol/kg **3o**. [h] Reference 32. **$p < 0.05$.

Table 2. Pharmacokinetic Parameters upon Oral Dosing of PDHK Inhibitors

pharmacokinetic parameters	**3i** (100 μmol/kg) average (n = 4)	**3k** (300 μmol/kg) average ± SD (n = 4)	**3o** (30 μmol/kg) average ± SD (n = 4)
$t_{1/2}$ (h)	4.6		3.0 ± 0.72
AUC (μM·h)$_{0-24}$	143_{0-8}	670 ± 301	196 ± 51.7
T_{max} (h)	0.5	6.0 ± 2.31	1.25 ± 0.5
C_{max} (μM)	87.3	53.8 ± 14.2	23.8 ± 1.42

In addition to the probable reduced toxicity due to not having halogens α to a carbonyl, these compounds are more potent in the primary enzymatic assay than any of the previously reported compounds by up to 500-fold. They are the first compounds other than dichlorinated halogenated acids known to be active in a cellular assay. The amides **3i**,**k**,**o**,**r** are potent and orally bioavailable inhibitors of PDHK in vivo. The expected consequence of PDHK inhibition, the activation of the PDH complex, was observed indirectly in vivo by measuring the lowering of lactate in normal 24-h fasted rats after oral dosing. These compounds will allow further pharmacological investigation of the effect of increasing oxidative disposal of lactate and pyruvate in disease states such as diabetes, ischemia, endotoxic shock, hemorrhagic shock, lactic acidosis, and cardiac insufficiency. Studies pertaining to the further exploitation of the SAR, the mechanism, and the pharmacology of this class of inhibitors are ongoing and will be the topic of subsequent reports.

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Supporting Information Available: X-ray crystallographic data of **3q** and detailed experimental procedures for **2**, **3h**,**m**,**n**, **6**, and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Caterson, I. D.; Kerby, A. L.; Cooney, G. J.; Frankland, R.; Denyer, G. S.; Nicks, J.; Williams, P. F. Inactivation of Pyruvate Dehydrogenase Complex in Heart Muscle Mitochondria of Gold-Thioglucose-Induced Obese Mice is not Due to a Stable Increase in Activity of Pyruvate Dehydrogenase Kinase. *Biochem. J.* **1988**, *253*, 291−294.

(2) (a) Wieland, O.; Siess, E.; Schulze-Wethmar, F. H.; von Funcke, H. G.; Winton, B. Active and Inactive Forms of Pyruvate Dehydrogenase in Rat Heart and Kidney: Effect of Diabetes, Fasting, and Refeeding on Pyruvate Dehydrogenase Interconversion. *Arch. Biochem. Biophys.* **1971**, *143*, 593−601. (b) Denyer, G. S.; Lam, D.; Cooney, G. J.; Caterson, I. D. Effect of Starvation and Insulin In Vivo on the Activity of the Pyruvate Dehydrogenase Complex in Rat Skeletal Muscles. *FEBS Lett.* **1989**, *250*, 464−468. (c) Imura, M.; Takatani, O. Short-Term Regulation of Pyruvate Dehydrogenase Complex by Insulin. *Horm. Metab. Res.* **1981**, *13*, 267−272.

(3) Mondon, C. E.; Jones, I. R.; Azhar, S.; Hollenbeck, C. B.; Reaven, G. M. Lactate Production and Pyruvate Dehydrogenase Activity in Fat and Skeletal Muscle From Diabetic Rats. *Diabetes* **1992**, *41*, 1547−1554.

(4) Mandarino, L. J.; Consoli, A.; Kelly, D. E.; Reilly, J. J.; Nurjhan, N. Fasting Hyperglycemia Normalizes Oxidative and Nonoxidative Pathways of Insulin-Stimulated Glucose Metabolism in Noninsulin-Dependent Diabetes Mellitus. *J. Clin. Endocrinol. Metab.* **1990**, *71*, 1544−1551.

(5) Del Prato, S.; Bonadonna, R. C.; Bonora, E.; Gulli, G.; Solini, A.; Shank, M.; DeFronzo, R. A. Characterization of Cellular Defects of Insulin Action in Type 2 (Non-Insulin-Dependent) Diabetes Mellitus. *J. Clin. Invest.* **1993**, *91*, 484−494.

(6) Henry, R. R.; Thorburn, A. W.; Beerdson, P.; Gumbiner, B. Dose−Response Characteristics of Impaired Glucose Oxidation in Non-Insulin-Dependent Diabetes Mellitus. *Am. J. Physiol.* **1991**, *261*, E132−E140.

(7) For a review of the structural components of the PDH multienzyme complex, see: Mattevi, A.; De Kok, A.; Perham, R. N. The Pyruvate Dehydrogenase Multienzyme Complex. *Curr. Opin. Struct. Biol.* **1992**, *2*, 877−887.
(8) Sugden, P. H.; Kerbey, A. L.; Randle, P. J.; Waller, C. A.; Reid, K. B. Amino Acid Sequences Around the Sites of Phosphorylation in the Pig Heart Pyruvate Dehydrogenase Complex. *Biochem. J.* **1979**, *181*, 419−426.
(9) Yeaman, S. J.; Hutcheson, E. T.; Roche, T. E.; Pettit, F. H.; Brown, J. R.; Reed, L. J.; Watson, D. C.; Dixon, G. H. Sites of Phosphorylation on Pyruvate Dehydrogenase from Bovine Kidney and Heart. *Biochemistry* **1978**, *17*, 2364−2370.
(10) (a) Popov, K. M.; Kedishvili, N. Y.; Zhao, Y.; Shimomura, Y.; Crabb, D. W.; Harris, R. A. Primary Structure of Pyruvate Dehydrogenase Kinase Establishes a New Family of Eukaryotic Protein Kinases. *J. Biol. Chem.* **1993**, *268*, 26602−26606. (b) Popov, K. M.; Kedishvili, N. Y.; Zhao, Y.; Gudi, R.; Harris, R. A. Molecular Cloning of the p45 Subunit of Pyruvate Dehydrogenase Kinase. *J. Biol. Chem.* **1994**, *269*, 29720−29724. (c) Gudi, R.; Bowker-Kinley, M. M.; Kedishvili, N. A.; Zhao, Y.; Popov, K. M. Diversity of the Pyruvate Dehydrogenase Kinase Gene Family in Humans. *J. Biol. Chem.* **1995**, *270*, 28989−28994.
(11) Linn, T. C.; Pettit, F. H.; Reed, L. J. α-Keto Acid Dehydrogenase Complexes. X. Regulation of the Activity of the Pyruvate Dehydrogenase Complex from Beef Kidney Mitochondria by Phosphorylation and Dephosphorylation. *Proc. Natl. Acad. Sci. U.S.A.* **1969**, *62*, 234−241.
(12) Cate, R. L.; Roche, T. E. A Unifying Mechanism for Stimulation of Mammalian Pyruvate Dehydrogenase$_a$ Kinase by Reduced Nicotinamide Adenine Dinucleotide, Dihydrolipoamide, Acetyl Coenzyme A, or Pyruvate. *J. Biol. Chem.* **1978**, *253*, 496−503.
(13) Randle, P. J.; Kerbey, A. L.; Espinal, J. Mechanisms of Decreasing Glucose Oxidation on Diabetes and Starvation: Role of Lipid Fuel and Hormones. *Diabetes Metab. Rev.* **1988**, *4*, 623−638.
(14) Pratt, M. L.; Roche, T. E. Mechanism of Pyruvate Inhibition of Kidney Pyruvate Dehydrogenase$_a$ Kinase and Synergistic Inhibition by Pyruvate and ADP. *J. Biol. Chem.* **1979**, *254*, 7191−7196.
(15) Whitehouse, S.; Cooper, R. H.; Randle, P. J. Mechanism of Activation of Pyruvate Dehydrogenase by Dichloroacetate and other Halogenated Carboxylic Acids. *Biochem. J.* **1974**, *141*, 761−774.
(16) Stacpoole, P. W.; Moore, G. W.; Kornhauser, D. M. Metabolic Effects of Dichloroacetate in Patients with Diabetes Mellitus and Hyperlipoproteinemia. *N. Engl. J. Med.* **1978**, *298*, 526−530.
(17) (a) Curry, S. H.; Chu, P. I.; Baumgartner, T. G.; Stacpoole, P. W. Plasma Concentrations and Metabolic Effects of Intravenous Sodium Dichloroacetate. *Clin. Pharmacol. Ther.* **1985**, *37*, 89−93. (b) Wells, P. G.; Moore, G. W.; Rabin, D.; Wilkinson, G. R.; Oates, J. A.; Stacpoole, P. W. Metabolic Effects and Pharmacokinetics of Intravenously Administered Dichloroacetate in Humans. *Diabetologia* **1980**, *19*, 109−113.
(18) Bersin, R. M.; Stacpoole, P. W. Dichloroacetate as Metabolic Therapy for Myocardial Ischemia and Failure. *Am. Heart J.* **1997**, *134*, 841−855.
(19) Preiser, J. C.; Moulart, D.; Vincent, J. L. Dichloroacetate Administration in the Treatment of Endotoxic Shock. *Circ. Shock* **1990**, *36*, 221−228.
(20) Granot, H.; Steiner, I. Successful Treatment of Irreversible Hemorrhagic Shock in Dogs with Fructose-1,6-Diphosphate and Dichloroacetate. *Circ. Shock* **1985**, *15*, 163−173.
(21) Henderson, G. N.; Curry, S. H.; Derendorf, H.; Wright, E. C.; Stacpoole, P. W. Pharmacokinetics of Dichloroacetate in Adult Patients with Lactic Acidosis. *J. Clin. Pharmacol.* **1997**, *37*, 416−425.
(22) Bersin, R.; Kwasman, M.; DeMarco, T.; Lau, D.; Klinski, C.; Golonka, J.; Chatterjee, K. Improved Hemodynamic Function in Congestive Heart Failure with the Metabolic Agent Sodium Dichloroacetate (DCA). *J. Am. Coll. Cardiol.* **1990**, *15*, 157A.
(23) Dichloroacetic acid showed efficacy in these indications. For a review of the pharmacology of DCA, see: Stacpoole, P. W. The Pharmacology of Dichloroacetate. *Metabolism* **1989**, *38*, 1124−1144.
(24) Stacpoole, P. W.; Moore, G. W.; Kornhauser, D. M. Toxicity of Chronic Dichloroacetate. *N. Engl. J. Med.* **1979**, *300*, 372.
(25) Yount, E. A.; Felten, S. Y.; O'Connor, B. L.; Peterson, R. G.; Powell, R. S.; Yum, M. N.; Harris, R. A. Comparison of the Metabolic and Toxic Effects of 2-Chloropropionate and Dichloroacetate. *J. Pharmacol. Exp. Ther.* **1982**, *222*, 501−508.
(26) Katz, R.; Tai, C. N.; Diener, R. M.; McConnell, R. F.; Semonick, D. E. Dichloroacetate, Sodium: 3-Month Oral Toxicity Studies in Rats and Dogs. *Toxicol. Appl. Pharmacol.* **1987**, *57*, 273−287.
(27) Toth, G. P.; Kelty, K. C.; George, E. L.; Read, E. J.; Smith, M. K. Adverse Male Reproductive Effects Following Subchronic Exposure of Rats to Sodium Dichloroacetate. *Fundam. Appl. Toxicol.* **1992**, *19*, 57−63.
(28) Halpert, J. R.; Balfour, C.; Miller, N. E.; Kaminsky, L. S. Dichloromethyl Compounds as Mechanism-Based Inactivators of Rat Liver Cytochromes P 450 In Vitro. *Mol. Pharmacol.* **1986**, *30*, 19−24.
(29) Bookstaff, R. C.; Moore, R. W.; Ingall, G. B.; Peterson, R. E. Androgenic Deficiency in Male Rats Treated with Perfluorodecanoic acid. *Toxicol. Appl. Pharmacol.* **1990**, *104*, 322−333.
(30) Espinal, J.; Leesnitzer, T.; Hassman, A.; Beggs, M.; Cobb, J. Inhibition of Pyruvate Dehydrogenase Kinase by Halogenated Acetophenones. *Drug Devel. Res.* **1995**, *35*, 130−136.
(31) Jackson, J. C.; Vinluan, C. C.; Dragland, C. J.; Sundararajan, V.; Yan, B.; Gounarides, J. S.; Nirmala, N. R.; Topiol, S.; Ramage, P.; Blume, J. E.; Aicher, T. D.; Bell, P. A.; Mann, W. R. Heterologously Expressed Inner Lipoyl Domain of Dihydrolipoyl Acetyltransferase Inhibits ATP-Dependent Inactivation of Pyruvate Dehydrogenase Complex: Identification of Important Amino Acid Residues. *Biochem. J.* **1998**, *334*, 703−711.
(32) Ohnmacht, C. J.; Russell, K.; Empfield, J. R.; Frank, C. A.; Gibson, K. H.; Mayhugh, D. R.; McLaren, F. M.; Shapiro, H. S.; Brown, F. J.; Trainor, D. A.; Ceccarelli, C.; Lin, M. M.; Masek, B. B.; Forst, J. M.; Harris, R. J.; Hulsizer, J. M.; Lewis, J. J.; Silverman, S. M.; Smith, R. W.; Warwick, P. J.; Kau, S. T.; Chun, A. L.; Grant, T. L.; Howe, B. B.; Li, J. H.; Trivedi, S.; Halterman, T. J.; Yochim, C.; Dyroff, M. C.; Kirkland, M.; Neilson, K. L. *N*-Aryl-3,3,3-trifluoro-2-hydroxy-2-methylpropanamides: K_{ATP} Potassium Channel Openers. Modifications on the Western Region. *J. Med. Chem.* **1996**, *39*, 4592−4601.
(33) (a) Brieden, W.; Naughton, A.; Robins, K.; Shaw, N.; Tinschert, A.; Zimmermann, T. Enzymic Method for the Stereoselective Preparation of (*S*)- or (*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acids. Ger. Offen. (b) Konigsberger, K.; Prasad, K.; Repic, O. The Synthesis of (*R*)- and (*S*)-α-Trifluoromethyl-α-hydroxycarboxylic Acids via Enzymatic Resolutions. *Tetrahedron Asymmetry* **1999**, *10*, 679−687.
(34) For the enzymatic resolution of the (*S*)-enantiomer, see: Crosby, J.; Holt, R. A.; Pittam, J. D. Enzymatic Process for Stereoselective Preparation of Therapeutic Amides. WO9738124-A 97.10.16 (9744).
(35) Kelly, S. E.; LaCour, T. G. A One Pot Procedure For the Synthesis of α-Hydroxyamides from the Corresponding α-Hydroxyacids. *Synth. Commun.* **1992**, *22*, 859−869.
(36) Desai, M.; Watthey, J. W. H.; Zuckerman, M. A Convenient Preparation of 1-Aroylpiperazines. *Org. Prep. Proced. Int.* **1976**, *8*, 85−86.
(37) (a) Bolos, J.; Gubert, S.; Anglada, L.; Planas, J. M.; Burgarolas, C.; Castello, J. M.; Sacristan, A.; Ortiz, J. A. 7-[3-(1-Piperidinyl)-propoxy]chromenones as Potential Atypical Antipsychotics. *J. Med. Chem.* **1996**, *39*, 2962−2970. (b) Morikawa, A.; Sone, T.; Asano, T. 5-Isoquinolinesulfonamide Derivatives. III. Synthesis and Vasodilatory Activity of 1-(5-Isoquinolinesulfonyl)piperazine Derivatives. *Chem. Pharm. Bull.* **1992**, *40*, 770−773.
(38) For the use of the 2-nitrobenzenesulfonyl moiety as a protecting group for amines, see: (a) Fukuyama, T.; Jow, C.-K.; Cheung, M. 2- and 4-Nitrobenzenesulfonamides: Exceptionally Versatile Means for Preparation of Secondary Amines and Protection of Amines. *Tetrahedron Lett.* **1995**, *36*, 6373−6374. (b) Fukuyama, T.; Cheung, M.; Jow, C.-K.; Hidai, Y.; Kan, T. 2,4-Dinitrobenzenesulfonamides: A Simple and Practical Method for the Preparation of a Variety of Secondary Amines and Diamines. *Tetrahedron Lett.* **1997**, *38*, 5831−5834.
(39) Craig, J. C.; Young, R. J. 1-Benzylpiperazine. *Organic Syntheses*; Wiley: New York, 1962; *Collect. Vol. V*, pp 88−91.
(40) Available from Sigma Aldrich Corp., Milwaukee, WI (cat.# P-7032).
(41) Inhibition of cAMP-dependent protein kinase was determined using a colorimetric cAMP-dependent protein kinase (PKA) assay kit (Pierce, Rockford, IL; cat.# 26500, SpinZyme format).
(42) Young, P. R.; McLaughlin, M. M.; Kumar, S.; Kassis, S.; Doyle, M. L.; McNulty, D.; Gallagher, T. F.; Fisher, S.; McDonnell, P. C.; Carr, S. A.; Huddleston, M. J.; Seibel, G.; Porter, T. G.; Livi, G. P.; Adams, J. L.; Lee, J. C. Pyridinyl Imidazole Inhibitors of p38 Mitogen-activated Protein Kinase Bind in the ATP site. *J. Biol. Chem.* **1997**, *272*, 12116−12121.
(43) Stock, J. B.; Ninfa, A. J.; Stock, A. M. Protein Phosphorylation and Regulation of Adaptive Responses in Bacteria *Microbiol. Rev.* **1989**, *53*, 450−490.
(44) An NMR structural determination of the catalytic domain of a prokaryotic histidine protein kinase has been completed; see: Tanaka, T.; Saha, S. K.; Tomomori, C.; Ishima, R.; Liu, D.; Tong, K. I.; Park, H.; Dutta, R.; Qin, L.; Swindells, M. B.; Yamazaki, T.; Ono, A. M.; Kainosho, M.; Inouye, M.; Ikura, M. NMR Structure of the Histidine Kinase Domain of the E. coli Osmosensor EnvZ. *Nature* **1998**, *396*, 88−92.
(45) Hanks, S. K. Eukaryotic Protein Kinases. *Curr. Opin. Struct. Biol.* **1991**, *1*, 369−383.
(46) The SAR of the anilides and the design strategy used to optimize them will be discussed in a full report; see: Aicher, T. D.;

Bebernitz, G. R.; Stanton, J.; Gao, J.; Shetty, S, S.; Knorr, D. C.; Strohschein, R. J.; Tan, J.; Liu, C.; Brand, L. J.; Yang, W. H.; Vinluan, C. C.; Kaplan, Emma; Dragland, C.; Islam, A.; Lozito, R. J.; Liu, X.; DelGrande, D.; Mann, W. R. Anilides of (*R*)-3,3,3-Trifluoro-2-hydroxy-2-methylpropionic acid as Inhibitors of Pyruvate Dehydrogenase Kinase. Manuscript in preparation.

(47) The SAR of the piperazines and the design strategy to optimize them will be presented in a full report; see: Aicher, T. D.; Anderson, R. C.; Gao, J.; Shetty, S. S.; Coppola, G. M.; Stanton, J. L.; Knorr, D. C.; Sperbeck, D. M.; Brand, L. J.; Vinluan, C. C., Kaplan, E. L.; Dragland, C.; Tomaselli, H. C.; Islam, A.; Lozito, R. J.; Liu, X.; Maniara, W. M.; Fillers, W. S.; DelGrande, D.; Walter, R. E.; Mann, W. R. Secondary Amides of (*R*)-3,3,3-Trifluoro-2-hydroxy-2-methylpropionic acid as Inhibitors of Pyruvate Dehydrogenase Kinase. Manuscript in preparation.

(48) Ofenstein, J. P.; Kiechle, F. L.; Dandurand, D. M.; Belknap, W. M.; Moore, K. H.; Holmes, R. D. Lactate Oxidation for the Detection of Mitochondrial Dysfunction in Human Skin Fibroblasts. *Anal. Biochem*. **1993**, *210*, 332−336.

(49) Glucose lowering was not expected to be evident in normal animals on the basis of Stacpoole's report (ref 17) and was not observed with compounds of this series in normal animals.

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