Anilides of (*R*)-Trifluoro-2-hydroxy-2-methylpropionic Acid as Inhibitors of Pyruvate Dehydrogenase Kinase

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The optimization of a series of anilide derivatives of (*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid as inhibitors of pyruvate dehydrogenase kinase (PDHK) is described that started from *N*-phenyl-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide **1** ($IC_{50} = 35 \pm 1.4$ μM). It was found that small electron-withdrawing groups on the ortho position of the anilide, i.e., chloro, acetyl, or bromo, increased potency 20−40-fold. The oral bioavailability of the compounds in this series is optimal (as measured by AUC) when the anilide is substituted at the 4-position with an electron-withdrawing group (i.e., carboxyl, carboxyamide, and sulfoxyamide). *N*-(2-Chloro-4-isobutylsulfamoylphenyl)-(*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionamide (**10a**) inhibits PDHK in the primary enzymatic assay with an IC_{50} of 13 ± 1.5 nM, enhances the oxidation of [^{14}C]lactate into $^{14}CO_2$ in human fibroblasts, lowers blood lactate levels significantly 2.5 and 5 h after oral doses as low as 30 μmol/kg, and increases the ex vivo activity of PDH in muscle, kidney, liver, and heart tissues. However, in contrast to sodium dichloroacetate (DCA), these PDHK inhibitors did not lower blood glucose levels. Nevertheless, they are effective at increasing the utilization and disposal of lactate and could be of utility to ameliorate conditions of inappropriate blood lactate elevation.

Introduction

The activity of the pyruvate dehydrogenase complex (PDH) is lower during conditions of reduced oxidative glucose metabolism such as obesity, starvation, and diabetes.[5−10] and in patients with congenital lactic acidosis. PDH activity is regulated by reversible phosphorylation. ATP-dependent phosphorylation of a specific serine residue of the E1 subunit of the pyruvate dehydrogenase complex (PDC) leads to its inactivation, and dephosphorylation reactivates the complex.[11−14]

Oral administration of sodium dichloroacetate (DCA), a known inhibitor of PDHKs, to type 2 diabetic patients lowered fasting plasma lactate, alanine, and glucose levels.[15−17] Although infusion of DCA lowered plasma lactate and alanine levels in healthy volunteers, no hypoglycemic effect was observed.[18] In addition to diabetes, DCA has proven efficacy as a therapy for ischemia,[19] endotoxic shock,[20] hemorrhagic shock,[21] lactic acidosis,[22] and cardiac insufficiency.[23,24] However, DCA cannot be used in long-term treatment due to toxicity. Until our recent publications,[25−27] no compounds other than α-dihalogenated carbonyl compounds were known to inhibit PDHK (see Figure 1).[28] Herein is reported the structural features of anilides of (*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid required for potent inhibition of PDHK in vitro and in vivo.

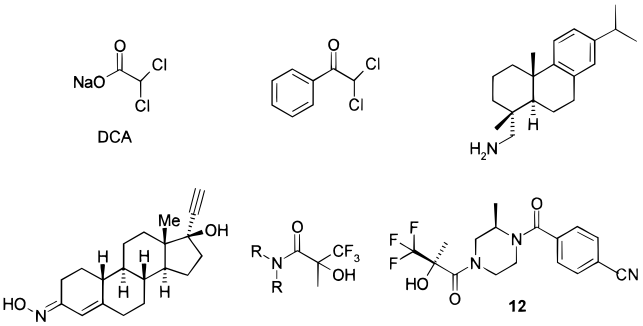

Figure 1. Known inhibitors of PDHK.

Chemistry

(*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid is most suitably prepared by the enzymatic resolution of the butyrate ester of the racemic acid.[29] The desired anilides were prepared by several methods, as outlined in Scheme 1. Utilizing the procedure of Morris et al.,[30] the anilides could be produced via treatment of the carboxylic acid with thionyl chloride (method A), followed by addition of the aniline.[31] The method affords high yields with anilines bearing electron-donating substituents, but affords poor yields of anilides when the aniline is 2,6-disubstituted. Some anilides were produced from isocyanides and 1,1,1-trifluoroacetone via the procedure of Seebach et al. (method B).[32] However, a modification of Kelly's procedure was the most general and effective synthetic method for producing the anilides (method C).[33] In short, the acyl halide was prepared by treating the α-hydroxycarboxylic acid with bis-(trimethylsilyl)urea and subsequent treatment with

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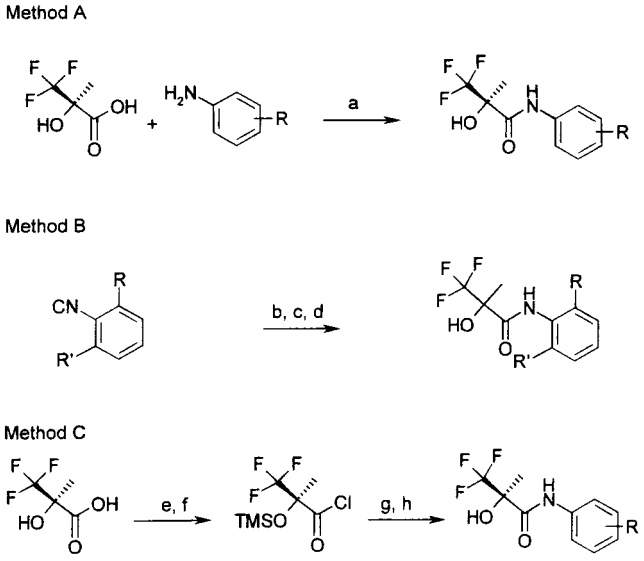
Scheme 1. General Methods of Synthesis of Anilide Analogues[a]



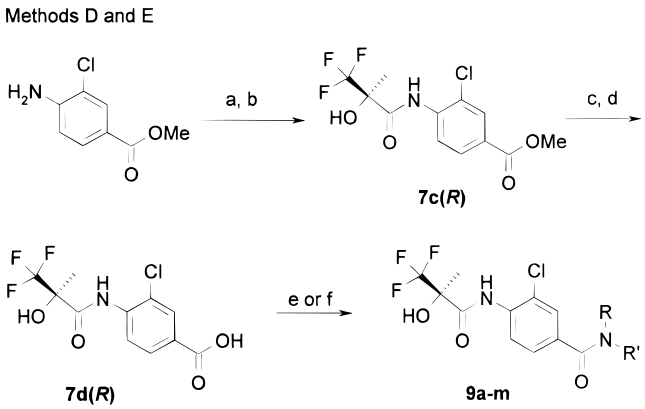
[a] Conditions: (a) $SOCl_2$, DMA; (b) $TiCl_4$, CH_2Cl_2; (c) CH_3COCF_3; (d) aq HCl; (e) 1,3-bis(trimethylsilylurea), CH_2Cl_2; (f) $(COCl)_2$, CH_2Cl_2, cat. DMF; (g) Et_3N, CH_2Cl_2, aniline; (h) aq HCl, MeOH.

oxalyl chloride in the presence of a catalytic amount of DMF. In the case of (*S*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionyl chloride, produced from (*R*)-3,3,3-trifluoro-2-(trimethylsiloxy)-2-methylpropionic acid, the acid chloride could 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 dilute methanolic hydrochloric acid.

The aniline precursors not commercially available were prepared via literature procedures. The 2-chloro-4-substituted anilines (i.e., 4-carbomethoxy, 4-benzoyl) were synthesized by treatment of the 4-substituted aniline with *N*-chlorosuccinimide by analogy to the method of Roche-Dolson.[34] 4-Chloro-5-nitrobenzophenone, 2-chloro-3-nitrobenzophenone (prepared via Friedel Crafts reaction of 3-nitro-2-chloro-benzoyl chloride with benzene), and methyl 2-chloro-3-nitrobenzoate were reduced to the anilines with stannous chloride via a modification of the procedure of Stille et al.[35] Methyl 4-chloro-3-nitrobenzoate was reduced to the corresponding aniline via a modification of the procedure of Hadley et al.[36]

The 2-chloro-4-carboxamide series was prepared (see Scheme 2) from the corresponding benzoate. Standard protocols were employed to couple the carboxylic acids (**6d**, **7d**, or **8d**) to the respective amines (method D, PyBOP; method E, EDC). During the synthesis of **9a**−**c**, and **9j**−**l**, the corresponding ethyl esters of the amino acids were coupled using method E followed by NaOH cleavage to afford the respective products. General methods for the preparation of the sulfonamides using solution phase chemistry are shown in Scheme 3. The sulfonamides **10a**−**z** were synthesized employing method F via coupling of the chiral sulfonylfluoride **7l(*R*)** with the appropriate amine. The destrifluoromethyl sulfonamide analogues **11a**−**d** could not be prepared in good yield using the standard protocol just illustrated, as 2-(trimethylsiloxy)-2-methylpropionyl chloride decomposed during the coupling reaction. A modified route

Scheme 2. General Methods of Synthesis of Carboxamide Analogues[a]



[a] Conditions: (a) (*S*)-trifluoro-2-(trimethylsiloxy)-2-methylpropionyl chloride, Et_3N, CH_2Cl_2; (b) aq HCl, MeOH; (c) aq KOH, MeOH; (d) aq HCl; (e) PyBOP, *N*-methylmorpholine, CH_2Cl_2, amine (method D); (f) EDC, DMAP, amine, DMF (method E).

(method G) was developed to prepare these analogues, via coupling to the commercially available 2-acetoxy-2-methylpropionyl chloride.

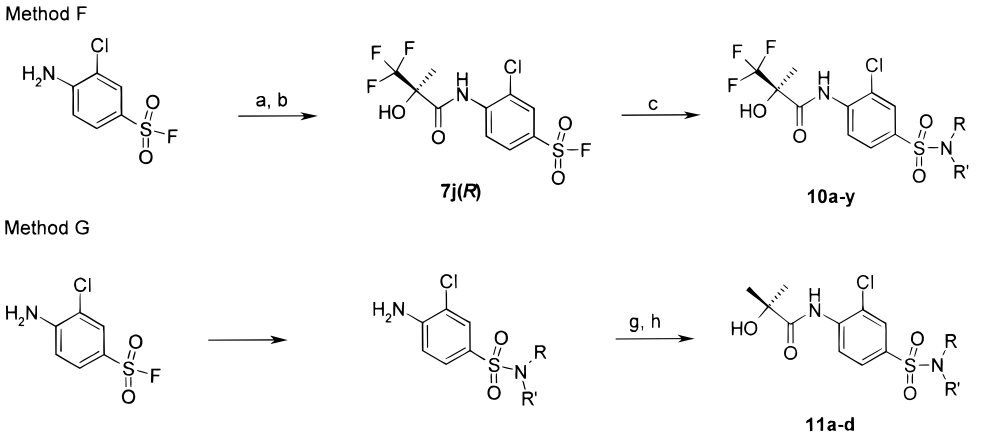
Results and Discussion

We previously reported that the amide **1** inhibited PDHK in the primary enzymatic assay.[25,27,37] 4-Substituted anilides 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.[38] It was demonstrated in amides of primary amines, secondary amines, and anilines that the (*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic moiety was greater than 40-fold more potent than the (*S*)-enantiomer and was the optimal acyl substituent for PDHK inhibitors.[27]

In the absence of any detailed structural information regarding PDHK, optimization of the aniline ring was accomplished by systematically varying the substituents at the ortho, meta, and para positions. To optimize the degree of diversity in the initial screening set, the first series of anilides (**2a**−**f**, **3a**−**h**, and **4a**−**f**) was selected on the basis of the principal components analysis of the aromatic substituents.[39] In addition, a limited number of 2,6-disubstituted analogues (**5a**−**c**) were synthesized in anticipation that substitution close to the (*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic moiety would have the most significant effect on potency.

The compounds were tested in the primary high-throughput assay, which utilizes the commercially available porcine PDH complex.[27,37,40] As expected, substitution at the ortho position most influenced activity due to its proximity to the pharmacophore. Of the initial six ortho-substituted compounds tested, the sterically small electron-withdrawing chlorine-substituted compound was clearly superior to the others (compare **2f** to **2a**−**e**). Consequently, analogues with other small substituents at the ortho position, most of them electron-withdrawing (i.e., **2g**−**m**), were profiled in an attempt to find an additional increase in potency. Overall, compounds with small electron-withdrawing substituents similar to the chloride analogue (**2f**) were also preferred, such as bromine (**2l**), nitro (**2k**), or acetyl (**2j**),

Scheme 3. General Methods of Synthesis of Sulfonamide Analogues[a]



[a] Conditions: (a) (*S*)-trifluoro-2-(trimethylsiloxy)-2-methylpropionyl chloride, Et_3N, CH_2Cl_2; (b) aq HCl, MeOH; (c) pyridine amine, CH_3CN; (d) pyridine, amine, DMAP; (e) HCl, EtOH, reflux; (f) amine, CH_3CN, reflux; (g) pyridine, DMAP, 2-acetoxy-2-methylpropionyl chloride, CH_2Cl_2; (h) aq NaOH, MeOH.

providing an increase in potency of up to 35-fold over the parent anilide **1**. Sterically demanding substituents (**2a**−**d**) or substituents able to donate hydrogen bonds at the 2-position (**2i**) were less potent or inactive.

Although monosubstitution at the 3- and 4-positions provided less dramatic increases in potency than 2-chloro substitution, trends could be detected. In general, electron-withdrawing substituents at either the 3- or 4-position increased potency 6−10-fold; i.e., 3- or 4-substitution with −COPh (compare **3d** and **4d** with **1**). Compounds with other electron-withdrawing substituents such as sulfones or sulfonamides at the 4-position were also more potent than the parent anilide **1**.

The inhibitors **2f**, **3d**, and **4a** 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 the previously described modification of Ofenstein's assay (Table 1).[27,41] It was encouraging that with these compounds, and in general with most of the later compounds, the order of potency in the cellular assay mirrored the order of potency in the primary enzymatic assay. Interestingly, the maximal response (efficacy) also increased, i.e., **4a** only maximally increased $^{14}CO_2$ production 3−4-fold while **2g** and **3d** maximally increased $^{14}CO_2$ production 8−10-fold. This degree of increase of $^{14}CO_2$ production proved to be the maximal amount.

Having obtained an initial increase in the enzymatic and cellular potency of this series, an effort was undertaken to evaluate the more potent monosubstituted anilides in vivo. They were profiled for their ability to lower lactate, the most proximal effect of PDHK inhibition in 24-h fasted Sprague−Dawley rats.[27] In the initial profiling of the monosubstituted anilide analogues, neither **2f** nor **4d** decreased blood lactate levels in vivo as expected for a PDHK inhibitor. Consequently, an investigation of the fate of **2f** after oral dosing in rats was undertaken by monitoring plasma levels. Inhibitor **2f** was quickly absorbed, reaching a C_{max} at 30 min.[42] However, it was rapidly metabolized and cleared. Importantly, no hydrolysis, conjugation, or metabolism was noted at the amide bond pharmacophore. Aromatic oxidative metabolism, which was presumed (see below) to be a potential problem, indeed was occurring as evidenced by the formation of a metabolite with a molecular ion of M + 17. The oxidation which occurred, presumably at the 4-position to result in the formation of **7g**, was very rapid (nearly all drug substance in the plasma was oxidatively metabolized after 30 min). The lack of efficacy of **4d** could not be explained by lack of absorption, since **4d** yields significant and long-enduring smooth muscle relaxation on the bladder of animal models of incontinence.[38] Consequently, we continued our efforts in improving the potency and the overall distribution by blocking aromatic oxidation via studying the effects of disubstitution on the anilide template.

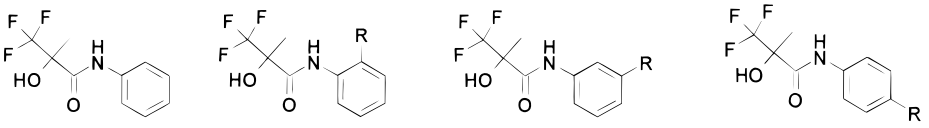
When profiling disubstituted anilides, the 2-Cl substituent was in general kept constant, as the 3-, 4-, and 5-substituents were systematically varied with carbonyl/carboxyl derivatives (see **6b**−**f**, **7b**−**f**, and **8b**−**f**). Three practical reasons dictated this choice of analogues. First, these substituents were generally readily synthetically accessible. Second, the positive effects of electron-withdrawing substituents at the 4- and 3(5)-positions for potency had been demonstrated (see above). Third, such compounds would be expected to decrease the propensity of the aromatic ring of the compounds to be oxidized upon oral dosing.

In general, 2,6-disubstitution or 2,5-disubstitution dramatically diminished the inhibition of PDHK (i.e., compare **5a** and **5b** to **2f** or compare **5c** to **2h** and compare **8b**−**f** to **2f** in Table 2), probably due to an unfavorable steric interaction. However, substitution at the 5-position with less sterically demanding substituents gave mixed results. The analogues **8a**, **8g**, and **8h** were approximately equal in potency to **2f**, while substitution at the 5-position with an electron-donating methoxy group resulted in an inactive analogue, **8i**.

However, substitution of **2f** at the 3- and 4-positions with carbonyl derivatives significantly improved the potency into the submicromolar range (i.e., compare **6b**, **6c**, **6f**, and **7b**−**e** to **2f**). A 6−10-fold difference in activity was observed in the amides substituted at the 3- and 4-positions respectively (compare **6e** to **6f**, or **7e** to **7f**), suggesting that an increase in potency could be achieved by modification of the amide group.

Three compounds [**7b(*R*)**, **7d(*R*)**, and **7f(*R*)**] of this initial set of disubstituted analogues were profiled in vivo. Each had significantly improved potency in the primary enzymatic assay and in the cellular assays.

Table 1. Physical Characteristics and in Vitro and in Vivo Data for Monosubstituted Anilide Analogues



1 **2a-m** **3a-h** **4a-h**

entry	R	mp (°C)	empirical formula[a]	% yield[b]	IC_{50} (μM)[c]	EC_{50} (μM)[d]
DCA[e]					>1000	130 ± 60
1		116	$C_{10}H_{10}NO_2F_3$	27	35.0 ± 1.4	
2a	CO_2Et	78−80	$C_{13}H_{14}NO_4F_3$	32	inactive	
2b	Ph	115	$C_{16}H_{14}NO_2F_3$	14	inactive	
2c	*t*-Bu	134	$C_{14}H_{18}NO_2F_3$	13	inactive	
2d	COPh	135−136	$C_{17}H_{14}NO_3F_3$	48	203 ± 7	
2e	OMe	122−123	$C_{11}H_{12}NO_3F_3$	28	61.5 ± 5.1	
2f	Cl	102−103	$C_{10}H_9NO_2ClF_3$	43	1.2 ± 0.4	5.40 ± 0.39
2g	F	88	$C_{10}H_9NO_2F_4$	42	4.15 ± 0.24	
2h	CH_3	117	$C_{11}H_{12}NO_2F_3$	48	16.9 ± 0.5	
2i	CH_2OH	153−155	$C_{11}H_{12}NO_3F_3$	27	145 ± 12	
2j	COMe	146−147	$C_{12}H_{12}NO_3F_3$	34	2.0 ± 0.5	14 ± 8.0
2k	NO_2	95	$C_{10}H_9N_2O_4F_3$	4	2.8 ± 0.4	
2l	Br	123	$C_{10}H_9NO_2BrF_3$	92	1.0 ± 0.1	
2m	CN	107	$C_{11}H_9N_2O_2F_3$	24	16.1 ± 1.2	
3a	OBn	83−84	$C_{17}H_{16}NO_3F_3$	28	22.2 ±1.6	
3b	NO_2	139	$C_{10}H_9N_2O_4F_3$	21	220 ± 19	
3c	CF_3	134−135	$C_{11}H_9NO_2F_6$	45	inactive	
3d	COPh	oil	$C_{17}H_{14}NO_3F_3$	4	3.0 ± 0.5	~10
3e	OMe	103−104	$C_{11}H_{12}NO_3F_3$	76	47.4 ± 5.2	
3f	CN	123−124	$C_{11}H_9N_2O_2F_3$	34	21.8 ± 0.9	
3g	COMe	155−156	$C_{12}H_{12}NO_3F_3$	50	170 ± 18	
3h	CH_2OH	oil	$C_{11}H_{12}NO_3F_3$	22	80 ± 15	
4a	OMe	115−116.5	$C_{11}H_{12}NO_3F_3$	52	22.6 ± 1.2	43.1 ± 9.6
4b	CO_2Et	144	$C_{13}H_{14}NO_4F_3$	14	17.7 ± 3.3	
4c	OPh	129	$C_{16}H_{14}NO_3F_3$*f*	20	42.1 ± 3.3	
4d	COPh	153	$C_{17}H_{14}NO_3F_3$	68	9.3 ± 0.8	8.27 ± 0.34
4e	Cl	113	$C_{10}H_9NO_2ClF_3$	11	17.3 ± 1.2	
4f	*t*-Bu	147	$C_{14}H_{18}NO_2F_3$	19	45.8 ± 3.2	
4g	SO_2NH_2	177−179	$C_{10}H_{11}N_2O_4F_3S$	17	6.4 ± 0.4	
4h	SO_2(1-piperidine)	166	$C_{15}H_{19}N_2O_4F_3S$	60	9.3 ± 0.4	

[a] Analytical results were within ±0.4% of the theoretical value. [b] Prepared via method A. [c] IC_{50} (μM ± standard error) in primary enzymatic assay of PDH kinase inhibition (ref 37). [d] EC_{50} (μM ± standard error) in cellular assay of increased oxidation of lactate (ref 27). [e] Sodium dichloroacetate. [f] C: calcd, 59.08; found, 58.51.

Although **7b(*R*)** and **7f(*R*)** did not decrease blood lactate levels in vivo, **7d(*R*)** was among the first (*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionamides to decrease blood lactate levels upon oral dosing.[27] To confirm that the in vivo efficacy was actually due to PDHK inhibition leading to activation of PDH complex activity, a previously described modification of Coore's ex vivo assay was employed in which the animal's tissues were removed after dosing and evaluated for PDH activity.[27,43] The ex vivo activity of the PDH complex was increased significantly in rat liver (2-fold) and muscle tissues (3-fold) after oral dosing of **7d(*R*)**.

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.[38] However, during our work, it was found that 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.[27] In addition, substitution at the 2-position of the anilide with a halogen has been reported to diminish the potency of K_{ATP} channel openers, while it markedly increases the potency of the PDHK inhibitors (i.e., compare **2a** to **2f** or **4d** to **7b**). Significantly, **7d(*R*)** presents no measurable effect on K_{ATP} activity in vitro (<100 μM) or in vivo (<300 μmol/kg/day), utilizing assays described in the literature.

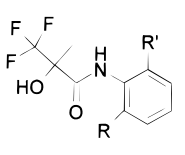
The above data suggested that 2-chloro-4-substituted anilides of (*R*)-trifluoro-2-hydroxy-2-methylpropionic acid were potentially orally active PDHK inhibitors. Thus our attention focused upon improving the pharmacological profile of these compounds via modification of the 4-substitutent. Toward this goal, libraries of 4-(carboxyamido)anilides **9a**−**m** and 2-chloro-4-(sulfonamido)anilides (**10a**−**y**) were prepared.

The library of 2-chloro-4-(carboxyamido)anilides **9a**−**m** provided a 16-fold range (see Table 3) of inhibitory activity in the primary enzymatic assay with the most potent inhibitor being the amide of 2-phenethylglycine **9c** (IC_{50} = 15 nM). Several of these amides were dosed (30 μmol/kg or 100 μmol/kg) in 24-h-fasted normal Sprague−Dawley rats. The cyclic secondary amides **9h**−**i** lowered blood lactate levels significantly 2 h after oral dosing. The acyclic secondary amides **9j**−**m** and the primary amides **9a**−**e** did not significantly lower blood lactate levels at the doses tested.

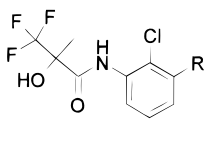
The sulfonamide anilides **10a**−**y** presented a 400-fold range of activity in the primary enzymatic assay. Most of these anilides possessed desirable potency in the primary enzymatic assay, and when tested at a concentration 10 times their IC_{50} in the cellular assay, they increased lactate conversion to CO_2 5−10-fold.

The four desfluoro derivatives (**11a**−**d**) of the sulfonamides (**10a**, **10g**, **10j**, and **10m**) were pursued to

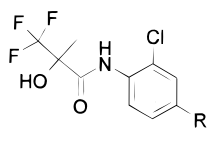
Table 2. Physical Characteristics and in Vitro and in Vivo Data for Disubstituted Anilide Analogues



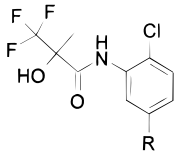
5a-5c



6a-f



7a-j



8a-i

entry	R	mp (°C)	empirical formula[a]	method	% yield	IC_{50} (μM)[b]	EC_{50} (μM)[c]	lactate[d] (% of control, μmol/kg)
DCA[e]						>1000	130 ± 60	70,* 1 mmol/kg
5a	Cl, Cl	159	$C_{10}H_8NO_2Cl_2F_3$	C	47	~30		
5b	Cl, Me	141−142	$C_{11}H_{11}NO_2ClF_3$	C	34	~60		
5c	Me, Me	172	$C_{12}H_{14}NO_2F_3$	B	23	inactive		
6a	Cl	120−1211	$C_{10}H_8NO_2Cl_2F_3$*f*	C	18	~0.3		
6b	COPh	122−123	$C_{17}H_{13}NO_3ClF_3$	A	47	0.380 ± 0.027	11.0 ± 1.7	
6c	CO_2Me	126−127	$C_{12}H_{11}NO_4ClF_3$	A	58	0.219 ± 0.035	13.8 ± 2.7	
6d	CO_2H	174.5−175.5	$C_{11}H_9NO_4ClF_3$	C	88	25 ± 3.6		
6e	CONH-*n*-hex	167−168	$C_{17}H_{22}N_2O_3ClF_3$	D	85	3.66 ± 0.33		
6f	CO(1-piperidine)	169−170	$C_{16}H_{18}N_2O_3ClF_3$	D	83	0.596 ± 0.037		
7a	Cl	131	$C_{10}H_8NO_2Cl_2F_3$	A	25	~2		
7b	COPh	140−142	$C_{17}H_{13}NO_3ClF_3$	A	35	0.113 ± 0.015	1−30 (sol?)	
7c	CO_2Me	119−121	$C_{12}H_{11}NO_4ClF_3$	A	16	0.115 ± 0.053		
				B	68			
7d(*R*)[g]	CO_2H	205−207	$C_{11}H_9NO_4ClF_3$		89	0.090 ± 0.015	1.40 ± 0.10	70,* 100 98, 30 μmol/kg
7e	CONH-*n*-hex	60−63	$C_{17}H_{22}N_2O_3ClF_3$*h*	D	81	0.126 ± 0.035	5.5 ± 1.7	
7f	CO(1-piperidine)	185−186	$C_{16}H_{18}N_2O_3ClF_3$	D	85	1.50 ± 0.12	0.71 ± 0.18	
7g	OH		$C_{10}H_9NO_3ClF_3$	C	49	0.56 ± 0.06		
7h	CN	207−208	$C_{11}H_8N_2O_2ClF_3$			0.15 ± 0.03		78,* 250 μmol/kg
7i(*R*)[g]	SO_2Me	165−166	$C_{10}H_{11}NO_4ClF_3S$			0.070 ± 0.010		65,* 250 μmol/kg
7j	SO_2F	137−139	$C_{10}H_8NSO_4ClF_4$	C	95	0.077 ± 0.010		
8a	Cl	142	$C_{10}H_8NO_2Cl_2F_3$	A	25	2.4 ± 0.44		
8b	COPh	195−196	$C_{17}H_{13}NO_3ClF_3$	A	12	inactive		
8c	CO_2Me	158−159	$C_{12}H_{11}NO_4ClF_3$	A	48	inactive		
8d	CO_2H	265−266	$C_{11}H_9NO_4ClF_3$		96	inactive		
8e	CONH-*n*-hex	148−149	$C_{17}H_{22}N_2O_3ClF_3$	D	98	inactive		
8f	CO(1-piperidine)	186−187	$C_{16}H_{18}N_2O_3ClF_3$	D	88	inactive		
8g	Me	139−140	$C_{11}H_{11}NO_2ClF_3$	C	50	3.8 ± 0.71		
8h	NO_2	167−168	$C_{10}H_8N_2O_4ClF_3$	C	28	2.7 ± 0.23		
8i	OMe	127−129	$C_{11}H_{11}NO_3ClF_3$	C	53	inactive		

[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 37). [c] EC_{50} (μM ± standard error) in cellular assay of increased oxidation of lactate (ref 27). [d] In vivo study in normal Sprague−Dawley rats (n = 6/group); animals are orally dosed (μmol/kg) after a 24-h fast. Lactate is expressed as percent of control, 2 h postdose (ref 27). [e] Sodium dichloroacetate. [f] H, N: calcd, 2.67, 4.64; found, 2.14, 4.11. [g] Synthesized from (*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid of 96% ee. [h] N: calcd, 7.10; found, 6.36. *p < 0.05.

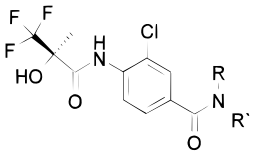
examine whether there was any benefit to not having the fluorines in the inhibitors. When optimizing the acyl moiety with 2-chloroaniline, the absence of the fluorines was less detrimental to the in vitro potency of the anilides than for the secondary amide series.[27] The results were as expected in terms of rank ordering based upon the potencies of **10a**, **10m**, **10p**, and **11g**, although the piperazine **11a** was considerably less potent than one would have predicted. The isobutylamine adduct **11c** was of sufficient potency that is was evaluated in vivo in comparison to its trifluoromethyl equivalent **10a** (see Table 4). It was less potent than **10a**; however, it did lower blood lactate levels at 300 μmol/kg.

Several of the sulfonamide anilides were profiled in vivo in 24-h-fasted Sprague−Dawley rats for their effect on blood lactate levels. All of the analogues tested lowered blood lactate levels significantly at either 2.5 and/or 5 h after oral dosing (i.e., see **10a**,**b**,**j**,**l**,**n** and others in Table 4). When profiling the levels of parent drug in plasma upon oral dosing, the sulfonamide **10j** exhibited its C_{max} at a later time than *N*-[3-(*R*)-methyl-4-(4-cyanobenzoyl)piperazine]-(*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (**12**) (see Figure 1) which has been previously reported.[27] In general, the sulfonamides effected a greater degree of lactate lowering 5 h after oral dose than at the 2.5 h after oral dose, which is consistent with the relatively slower rate of absorption of this class of compounds as compared to the piperazine **12** (see Table 5).

Having demonstrated that the PDHK inhibitors **10a**, **10j**, and **11c** are orally active; i.e., they activate the PDH complex at low doses in normal animals, the compounds' effects in disease models of type 2 diabetes were investigated. In Zucker diabetic rats, both **10a** and **10j** elevated PDH activity ex vivo (see Figure 2).[44] Both compounds lowered blood lactate levels significantly (>38%) at 2.5 and 5 h postdose in both the fasted and fed states in these diabetic rats. However, similar to the secondary amide **12**, no marked hypoglycemic effect was noted, nor was any marked effect seen in other animal models of type 2 diabetes.[27] In contrast to these findings, DCA treatment produced a hypoglycemic response. It has been shown elsewhere that DCA can also elicit direct effects on fatty acid oxidation by inhibition of short- and medium-chain acyltransferases. These direct effects may be relevant in explaining the observed effects of DCA on glucose levels.[45,46]

The mechanism by which this class of compounds

Table 3. Physical Characteristics and in Vitro and in Vivo Data for Disubstituted Anilide Analogues



9a-m

entry	amine = NRR′	mp (°C)	empirical formula[a]	% yield[b]	IC_{50} (nM)[c]	lactate[d] (% of control, μmol/kg)
DCA[e]					>1000000	70,* 1 mmol/kg
9a	(L)-phenylalanine-Na	170−172	$C_{20}H_{17}ClF_3N_2O_5{\cdot}Na$	69	34 ± 11	115, 100
9b	(L)-4-(phenyl)phenylalanine	96−98	$C_{26}H_{22}ClF_3N_2O_5$	65	77 ± 15	101, 30
9c	(L)-2-phenethylglycine	95−97	$C_{21}H_{20}ClF_3N_2O_5$	58	15 ± 15	97, 30
9d	6-aminovaleric acid-Na	165−167	$C_{17}H_{19}ClF_3N_2O_5.Na$	45	180 ± 31	94, 100
9e	3-hydroxypropylamine	oil	$C_{14}H_{16}ClF_3N_2O_4$	46	87 ± 12	88, 100
9f	3,4,5-trimethoxyaniline	110−112	$C_{20}H_{20}ClF_3N_2O_6$	50	160 ± 15	
9g	(rac)-tetrahydrofuran-2-yl)methylamine	100−103	$C_{16}H_{18}ClF_3N_2O_4$	77	240 ± 49	
9h	morpholine	179−180	$C_{15}H_{16}ClF_3N_2O_4$	75	130 ± 33	64,* 100
9i	2-pyridylpiperazine	92−94	$C_{20}H_{20}ClF_3N_4O_3$	59	110 ± 14	63,* 100
9j	(L)-*N*-Me alanine-Na	208−210	$C_{15}H_{15}ClF_3N_2O_5{\cdot}Na$	60	150 ± 50	94, 30
9k	(L)-*N*-Me-valine-Na	203−205	$C_{17}H_{19}ClF_3N_2O_5{\cdot}Na$	46	140 ± 29	87, 30
9l	(L)-*N*-Me-tryptophan-Me ester	95−98	$C_{24}H_{23}ClF_3N_3O_5$	100	110 ± 20	102, 30
9m	3-hydroxy-*N*-Me propylamine	oil	$C_{15}H_{18}ClF_3N_2O_4 1.0H_2O$	100	47 ± 5.7	114, 30

[a] Analytical results were within ±0.4% of the theoretical value. [b] Prepared via method E. [c] IC_{50} (μM ± standard error) in primary enzymatic assay of PDH kinase inhibition (ref 37). [d] In vivo study in normal Sprague−Dawley rats (n = 6/group); animals are orally dosed (μmol/kg) after a 24-h fast. Lactate is expressed as percent of control, 2.5 h postdose (ref 27). [e] Sodium dichloroacetate. *p < 0.05.

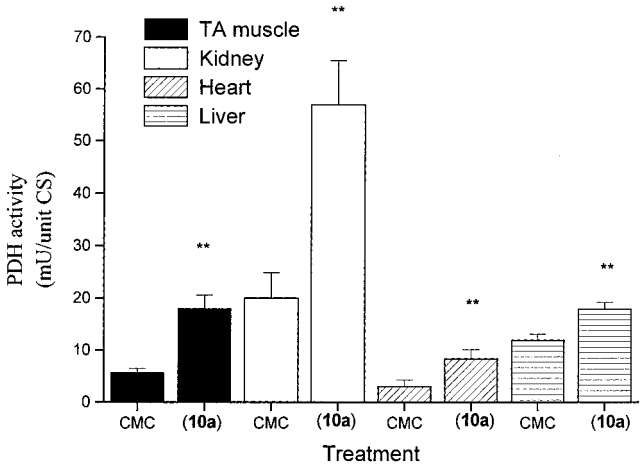


Figure 2. PDH complex activation by **10a** in diabetic zucker rats. Effect of **10a** on PDH activity in tibalis anterior muscle, kidney, liver, and heart tissues from 24-h fasted Zucker diabetic rats (100 μmol/kg/day, 11 days of dosing, 5 h after oral dose, n = 8 animals per group). The PDH complex activity was measured in an arylamine acetyltransferase-coupled spectrophotometric assay and was normalized for citrate synthase activity (see ref 27). **p < 0.05 vs the CMC treated group.

inhibits PDHK has been investigated and will be the subject of a future paper.[47] In brief, these compounds are not competitive with respect to ATP, but they appear to interfere with the interaction of PDHK with the PDH complex.

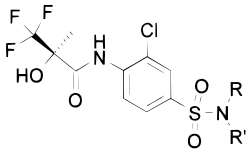
In conclusion, we report the identification of several anilides as orally available PDHK inhibitors. For example, **10a** inhibits PDHK in the primary enzymatic assay with an IC_{50} of 13 ± 1.5 nM, enhances the oxidation of [^{14}C]lactate into $^{14}CO_2$ in human fibroblasts, diminished blood lactate levels significantly 2.5 and 5 h after oral doses as low as 30 μmol/kg in normal and diabetic animals, and increases the ex vivo activity of PDH in muscle, kidney, liver, and heart tissues. However, these selective PDHK inhibitors did not lower blood glucose in animal models of type 2 diabetes. Nevertheless, they are effective at increasing the utilization and disposal of blood lactate and could be of utility to ameliorate pathological conditions of inappropriate blood lactate elevation.

Experimental Section

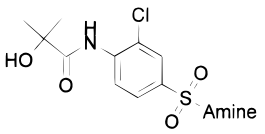
(a) Chemistry. All melting points (mp) were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton magnetic resonance spectra were recorded on a Bruker AC 300-MHz spectrometer. Chemical shifts were recorded in ppm (δ) and are reported relative to the solvent peak or 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 were of purified product and were not optimized. All starting materials were commercially available unless otherwise indicated.

General Procedures. Method A. *N*-(4-Methoxyphenyl)-3,3,3-trifluoro-2-hydroxy-2-methylpropionamide (4a). Thionyl chloride (1.53 mL, 21 mmol) was added dropwise for 30 min to a solution of 3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid (3.16 g, 20 mmol) in *N*,*N*-dimethylacetamide (20 mL) at −20 °C, and the mixture was stirred for an additional 10 min at −10 °C. *p*-Anisidine (2.50 g, 20.3 mmol) was added to the mixture, which then was stirred for an additional 90 min at room temperature. The mixture was concentrated to a paste and partitioned between ethyl acetate and water. The organic phase was dried (Na_2SO_4) and concentrated. Crystallization of the residue from ether/hexane afforded off-white crystals (2.9 g) which were dissolved in EtOAc and treated with charcoal, filtered through Celite, concentrated, and recrystallized from ether/hexane to afford **4a** (2.55 g, 52%) as white crystals: mp 115−116.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.72 (s, 3H), 3.81 (s, 3H), 3.86 (bs, 1H), 6.89 (d, J = 9.0 Hz, 2H), 7.44 (d, J = 9.0 Hz, 2H), 8.13 (bs, 1H); MS (DCI, NH_3) m/z (rel intensity) 265 (8), 264 (100), 210 (20). Anal. ($C_{11}H_{12}NO_3F_3$) C, H, N, F.

Table 4. Physical Characteristics and in Vitro and in Vivo Data for 2-Chloro-4-sulfonamide Anilide Analogues



10a-y



11a-d

entry	amine = NRR′	mp (°C)	empirical formula[a]	% yield[b]	IC_{50} (nM)[c]	lactate[d] (% of control μmol/kg) 2.5 h after oral dose	lactate[d] (% of control μmol/kg) 5 h after oral dose
DCA[e]					>1000000	70,* 1 mmol/kg	
10a	isobutylamine	141−142	$C_{14}H_{18}ClF_3N_2O_4S$	50	13 ± 1.5	69,* 100 83, 30 100, 10	60,* 100 67,* 30 95, 30
10b	allylamine	105−107	$C_{13}H_{14}ClF_3N_2O_4S$	56	8 ± 2.3	76,* 100	62,* 100
10c	diethylamine	100−102	$C_{14}H_{18}ClF_3N_2O_4S$	22	61 ± 11		
10d	3,3-dimethylbutylamine	144−145	$C_{16}H_{22}ClF_3N_2O_4S$	76	20 ± 3.1		
10e	aminomethylcyclopropane	130−131	$C_{14}H_{16}ClF_3N_2O_4S$	63	7.1 ± 2.8		
10f	cyclododecylamine	109−111	$C_{22}H_{32}ClF_3N_2O_4S$·0.2hex	75	380 ± 60		
10g	decylamine	67−68	$C_{20}H_{30}ClF_3N_2O_4S$	67	1300 ± 90		
10h	2-aminobicyclohexyl	98−104	$C_{22}H_{30}ClF_3N_2O_4S$·0.2hex	19	>3000		
10i	1,3-cyclohexanediamine	175−180	$C_{16}H_{21}ClF_3N_3O_4S$	42	8.4 ± 2.3		
10j	piperazine	227−228	$C_{14}H_{17}ClF_3N_3O_4S$	53	71 ± 8.3	77,* 300	70,* 300
10k	*N*-2-pyridylpiperazine	190−191	$C_{19}H_{20}ClF_3N_4O_4S$	51	73 ± 9.2		
10l	4-fluorophenethylamine	169−170	$C_{18}H_{17}ClF_4N_2O_4S$	87	35 ± 2.7	124, 100	69,* 100
10m	3,4,5-trimethoxybenzylamine	175−176	$C_{20}H_{22}ClF_3N_2O_7S$	69	130 ± 22		
10n	2-aminomethylpyridine	142−143	$C_{16}H_{15}ClF_3N_3O_4$	75	20 ± 3.7	64,* 100	84, 100
10o	3-aminomethylpyridine	oil	$C_{16}H_{15}ClF_3N_3O_4S$·0.9TFA	68	140 ± 19		
10p	4-(2-aminoethyl)morpholine	180−184	$C_{16}H_{21}ClF_3N_3O_5S$·HCl	65	10 ± 2.7	71,* 100	84, 100
10q	1-(2-aminoethyl)pyrrolidine	99−102	$C_{16}H_{21}ClF_3N_3O_4S$·1.0 TFA	29	920 ± 80		
10r	1-(3-aminopropyl)-2-pyrrolidinone	88−90	$C_{17}H_{21}ClF_3N_3O_5S$	59	16 ± 4	51,* 100 87, 30	52,* 100 66,* 30
10s	3-diethylaminopropylamine	68−72	$C_{17}H_{25}ClF_3N_3O_4S$·0.5 TFA	57	66 ± 25		
10t	4-amino-1-benzylpiperidine	122−125	$C_{22}H_{25}ClF_3N_3O_4S$·1.2 TFA	7	230 ± 28		
10u	3-aminopyrrolidine	125−127	$C_{14}H_{17}ClF_3N_3O_4S$·1.1 TFA	76	23 ± 4.1	82, 100	70,* 100
10v	4-(2-aminoethyl)benzene-sulfonamide	74−79	$C_{18}H_{19}ClF_3N_3O_6S$	30	16 ± 2.7	82, 100	87,* 100
10w	*N*,*N*-dimethylethylenediamine	128−130	$C_{14}H_{19}ClF_3N_3O_4S$·1.0 TFA	65	32 ± 6.6		
10x	3-ethoxypropylamine	oil	$C_{15}H_{20}ClF_3N_2O_5S$·0.2 EtOAc	84	19 ± 0.57	72,* 100	70,* 100
10y	3-hydroxypropylamine	115−118	$C_{13}H_{16}ClF_3N_2O_5S$	29	29 ± 1.7		
11a	piperazine	153−155	$C_{14}H_{20}ClN_3O_4S$	51[f]	1490 ± 995		
11b	decylamine	109−110	$C_{20}H_{33}ClN_2O_4S$	57[f]	4730 ± 840		
11c	isobutylamine	123−124	$C_{14}H_{21}ClN_2O_4S$	67[f]	98 ± 11	69,* 300 101, 100	76, 300 95, 100
11d	3,4,5-trimethoxybenzylamine	72−74	$C_{20}H_{25}ClN_2O_3S$	47[f]	1800 ± 120		

[a] Analytical results were within ±0.4% of the theoretical value. [b] All compounds were prepared by method F, except as noted. [c] IC_{50} (μM ± standard error) in primary enzymatic assay of PDH kinase inhibition (ref 36). [d] In vivo study in normal Sprague−Dawley rats (n = 6/group); animals are orally dosed (μmol/kg) after a 24-h fast. Lactate is expressed as percent of control (ref 27). [e] Sodium dichloroacetate. [f] Prepared by method G. *p < 0.05.

Table 5. Phamacokinetic Parameters upon Oral Dosing of PDHK Inhibitors

pharmacokinetic parameters	**6d** (100 μmol/kg)	**10j** (300 μmol/kg)	**12** (30 μmol/kg)[b]
$t_{1/2}$ (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

[a] All are average values for n = 4. [b] ±SD.

Method B. *N*-(2,6-Dimethylphenyl)-3,3,3-trifluoro-2-hydroxy-2-methylpropionamide (5c). A 1.0 M solution of titanium tetrachloride in CH_2Cl_2 (9.8 mL, 9.8 mmol) was added to a solution of 2,6-dimethylphenyl isocyanide (1.18 g, 9.0 mmol) in CH_2Cl_2 at 0 °C. After stirring for 1 h, 1,1,1-trifluoroacetone (1.0 g, 8.9 mmol) was added to the mixture. The reaction was stirred an additional 8 h, 1 N HCl (50 mL) was added, and the stirring was continued for 16 h. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried, and concentrated. The solid was recrystallized from a mixture of hexane and CH_2Cl_2 to afford **5c** (540 mg, 23%) as white crystals, mp 172 °C; ^{1}H NMR ($CDCl_3$) δ 1.76 (s, 3H), 2.12 (s, 6H), 4.03 (bs, 1H), 7.16−7.26 (m, 3H), 8.05 (bs, 1H); MS (DCI, NH_3) m/z (rel intensity) 263 (9), 262 (100). Anal. ($C_{12}H_{14}NO_2F_3$) C, H, N.

Method C. (*R*)-(+)-(2-Chloro-4-carboxymethylphenyl)-3,3,3-trifluoro-2-hydroxy-2-methylpropionamide (7c(*R*)). 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, and 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.

One-half of the (*S*)-3,3,3-trifluoro-2-(trimethylsiloxy)-2-methylpropionyl chloride solution in CH_2Cl_2 (10 mmol) was added to a solution of 2-chloro-4-carboxymethylaniline (1.85 g, 10 mmol) and Et_3N (4 mL, 29 mmol) in CH_2Cl_2 at 0 °C. The mixture was stirred for 16 h and concentrated to a paste. The crude silylated amide was hydrolyzed by the addition of MeOH (100 mL) and 2 N HCl (10 mL) and stirred overnight. The mixture was diluted with water (40 mL), concentrated to 50

mL, and then partitioned between Et_2O and 2 N HCl. The organic layer was dried ($MgSO_4$) and concentrated. The residue was filtered through silica gel with CH_2Cl_2. Hexane was added to the eluent to induce crystallization of **7c(*R*)** (1.78 g, 55%) as white crystals: mp 151.5−153 °C; ^{1}H NMR ($CDCl_3$) δ 1.79 (s, 3H), 3.55 (s, 1H), 3.94 (s, 3H), 8.00 (dd, J = 8.0, 0.9 Hz, 1H), 8.10 (d, J = 0.9 Hz, 1H), 8.53 (d, J = 8.0 Hz, 1H), 9.17 (bs, 1H); MS (DCI, NH_3) *m/z* (rel intensity) 328 (33), 327 (7), 326 (100). Anal. ($C_{12}H_{11}NO_4ClF_3$) C, H, N, F, Cl.

(*R*)-(+)-(2-Chloro-4-carboxyphenyl)-3,3,3-trifluoro-2-hydroxy-2-methylpropionamide (7d(*R*)). A solution of potassium hydroxide (1.12 g, 20 mmol) in water (15 mL) was added to a solution of **7c(*R*)** (1.58 g, 4.85 mmol) in methanol (40 mL), and the mixture stirred overnight. The mixture was cooled to 0 °C, and 2 N HCl was added (10.5 mL, 21 mmol). The resulting mixture was concentrated to 15 mL, diluted with water (30 mL), filtered, washed several times with water, and dried to afford the acid **7d(*R*)** (1.43 g, 95%) as white crystals: mp 218.5−222 °C, $[\alpha]_D$ = −1.44 (c = 1.02, MeOH); ^{1}H NMR (CD_3OD) δ 1.67 (s, 3H), 7.98 (dd, J = 8.6, 2.0 Hz, 1H), 8.08 (d, J = 2.0 Hz, 1H), 8.42 (d, J = 8.6 Hz, 1H); MS (DCI, isobutane) *m/z* (rel intensity) 314 (32), 313 (6), 312 (100). Anal. ($C_{11}H_9NO_4ClF_3$) C, H, N, F, Cl.

Method D. *N*-[2-Chloro-4-(piperidine-1-carbonyl)-phenyl]-(*R*)-(+)-3,3,3-trifluoro-2-hydroxy-2-methylpropionamide (7f(*R*)). (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) (208 mg, 0.4 mmol) was added to a mixture of **7d(*R*)** (124 mg, 0.40 mmol), piperidine (34 mg, 0.4 mmol), and *N*-methylmorpholine (88 ?L, 0.77 mmol) in CH_2Cl_2 (1 mL). The mixture was stirred for 4 h at room temperature. The crude mixture was filtered through SiO_2 (30 g) eluting with hexane:ethyl acetate (1:1). The elutant was concentrated, and the residue was crystallized from hexane:CH_2Cl_2 to afford **7f (*R*)** (128 mg, 85%) as white crystals: mp 185−6 °C; ^{1}H NMR ($CDCl_3$) δ 1.43−1.70 (m, 6H), 1.75 (s, 3H), 3.25−3.40 (bm, 2H), 3.62−3.82 (bm, 2H), 6.29 (s, 1H), 7.23 (d, J = 8.0 Hz, 7.36 (s, 1H), 8.34 (d, J = 8.0 Hz, 1H), 9.01 (bs, 1H); MS (DCI, NH_3) *m/z* (rel intensity) 381 (33), 379 (100). Anal. ($C_{16}H_{18}N_2O_3F_3Cl$) C, H, N, F, Cl.

Method E. *N*-[2-Chloro-4-(tetrahydrofuran-2-ylmethyl-amino-1-carbonyl)phenyl]-(*R*)-(+)-3,3,3-trifluoro-2-hydroxy-2-methylpropionamide (9g). *p*-(Dimethylamino)-pyridine (DMAP) (342 mg, 2.8 mmol) and 1-(3-dimethyamino-propyl)-3-ethylcarbodiimide hydrochloride (EDC) (306 mg, 1.6 mmol) were added to a solution of 3-chloro-4(*R*)-(+)-(3,3,3-trifluoro-2-hydroxy-2-methylpropionylamino)benzoic acid (**7d (*R*)**) (250 mg, 0.80 mmol) in DMF (5 mL), and the resulting mixture was stirred for 10 min, followed by the addition of *C*-(tetrahydrofuran-2-yl)methylamine (97 mg, 0.96 mmol). The mixture was stirred for 18 h, concentrated, and partitioned between EtOAc (15 mL) and 1 N HCl. The aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with 1 N HCl, 1 N $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated. The residue was subjected to column chromatography on silica gel eluting with hexane: ethyl acetate (9:1). The major fraction was crystallized from diethyl ether to afford **9g** (244 mg, 77%) as white crystals: mp 100−103 °C; ^{1}H NMR ($CDCl_3$) δ 1.6−2.2 (m, 4H), 1.75 (s, 3H), 3.3 (m, 1H), 3.7−3.9 (m, 3H), 4.1 (m, 1H), 5.27 (d, J = 17.2 Hz, 1H), 6.6 (m, 1H), 7.6 (m, 1H), 7.8 (dd, J = 4.5, 1.8 Hz, 1H), 8.43 (d, J = 8.5 Hz, 1H), 9.20 (d, J = 6.2 Hz, 1H); MS DCI *m/z* (rel intensity) 395 (100). Anal. ($C_{16}H_{18}N_2O_4F_3Cl$) C, H, N.

Method F. *N*-(2-Chloro-4-isobutylsulfamoylphenyl)-(*R*)-(+)-3,3,3-trifluoro-2-hydroxy-2-methylpropionamide (10a). 4-Amino-3-chlorobenzenesulfonyl fluoride (6.27 g, 30 mmol) was acylated according to method C, and the major product was recrystallized from methyl *tert*-butyl ether to afford **7l** (6.5 g, 62%) as white crystals: mp 137−139 °C; ^{1}H NMR ($CDCl_3$) δ 1.80 (s, 3H), 3.54 (s, 1H), 7.95 (dd; J = 2.1, 8.8 Hz; 1H), 8.07 (d, J = 2.2 Hz, 1H), 8.77 (d, J = 8.8 Hz, 1H), 9.48 (s, 1H); MS (DCI) *m/z* (rel intensity) 350 (100). Anal. ($C_{10}H_8ClF_4NO_4S$) C, H, N.

Isobutylamine (12.4 mg, 0.17 mmol) was added to a solution of **7j** (30 mg, 0.086 mmol) and DMAP (15 mg, 0.12 mmol) in acetonitrile (2 mL) and agitated for 18 h. The crude reaction mixture was added to the top of a 1 g silica gel SEP-PAK and eluted with acetonitrile (4 mL). The residue was recrystallized from toluene to provide **10a** (17 mg, 50%) as white crystals: mp 141−142 °C; ^{1}H NM (CD_3OD) δ 0.87 (d, J = 6.6 Hz, 6H), 1.68 (s, 3H), 1.69 (m, 1H), 2.65 (d, J = 6.6 Hz, 2H), 7.78 (dd, J = 8.7, 2.0 Hz, 1H), 7.93 (d, J = 2.0 Hz, 1H), 8.48 (d, J = 8.7 Hz, 1H); MS (M^-) *m/z* (rel intensity) 401.0 (100). Anal. ($C_{14}H_{18}ClF_3N_2O_4S$) C, H, N.

Alternate General Workup Procedures. For reactions with R_f (silica gel, 1:1 hexanes:ethyl acetate) of 0.2−0.9, the crude mixtures were added to the top of a 1 g SEP-PAK of silica and eluted with acetonitrile (4 mL), plus additional EtOAc (4 mL) for compounds with R_f of 0.4−0.9. For those compounds with R_f of less than 0.2, the reactions were partitioned between EtOAc and 1 N HCl (or water if an additional basic amine is present in the product) and extracted with EtOAc. The material was dried over $MgSO_4$ and evaporated to a crude oil followed by HPLC purification.

Method G. *N*-(2-Chloro-4-isobutylsulfamoylphenyl)-2-hydroxy-2-methylpropionamide (11c). Pyridine (10 mL, 125 mmol) and isobutylamine (5.2 g, 72 mmol) were added to a solution of 2-chloro-4-(fluorosulfonyl)aniline (5.0 g, 24 mmol) in acetonitrile (50 mL), and the mixture was stirred for 24 h. Additional isobutylamine (2.1 g, 30 mmol) was then added and the reaction stirred an additional 24 h. The reaction was concentrated and chromatographed on silica eluting with a gradient of hexane:ethyl acetate (4:1 to 1:1) to afford 4-amino-3-chloro-*N*-isobutylbenzenesulfonamide (4.5 g, 71%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.88 (d, J = 6.7 Hz, 6H), 1.71 (sextuplet, J = 6.7 Hz, 1H), 2.73 (t, J = 6.7 Hz, 2H), 4.3 (bs, 1H), 6.78 (d, J = 8.2 Hz, 1H), 7.53 (dd, J = 8.2, 2.2 Hz, 1H), 7.76 (d, J = 2.2 Hz, 1H); MS CI *m/z* (rel intensity) 261 *m/z* (rel intensity) MH^- (100).

2-Acetoxyisobutyryl chloride (950 mg, 5.7 mmol) was added to a solution of 4-amino-3-chloro-*N*-isobutylbenzenesulfonamide (1.0 g, 3.8 mmol) in 20% pyridine/CH_2Cl_2 (20 mL). The reaction was stirred for 3 h and then concentrated to a crude solid, which was partitioned between EtOAc and 1 N HCl. The aqueous layer was reextracted with EtOAc, and the combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated to afford acetic acid 1-(2-chloro-4-isobutylsulfamoylphenylcarbamoyl)-1-methylethyl ester as a crude solid (1.6 g): ^{1}H NMR ($CDCl_3$) δ 0.88 (d, J = 6.7 Hz, 6H), 1.57 (s, 6H), 1.71 (sextuplet, J = 6.7 Hz, 1H), 2.16 (s, 3H), 2.75 (t, J = 6.7 Hz, 2H), 4.56 (bs, 1H), 7.74 (dd, J = 8.2, 2.2 Hz, 1H), 7.89 (d, J = 2.2 Hz, 1H), 8.64 (d, J = 8.2 Hz, 1H), 8.7 (bs, 1H); MS CI 389 *m/z* (rel intensity) MH^- (100).

The crude amide was treated with 1 N NaOH (11 mL) in methanol (30 mL) for 3 h. $NaHCO_3$ (8 mL of 10% aq solution) was added and the reaction was concentrated until a solid precipitated from the aqueous solution and was filtered to afford **11c** as a white powder (1.3 g, 95%): mp 138−139 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (d, J = 6.7 Hz, 6H), 1.58 (s, 6H), 1.71 (sextuplet, J = 6.7 Hz, 1H), 2.53 (s, 1H), 2.73 (t, J = 6.7 Hz, 2H), 4.57 (t, J = 6.2 Hz, 1H), 7.72 (dd, J = 8.2, 2.2 Hz, 1H), 7.88 (d, J = 2.2 Hz, 1H), 8.65 (d, J = 8.2 Hz, 1H), 9.6 (bs, 1H); MS CI *m/z* (rel intensity) 347 MH^- (100). Anal. ($C_{14}H_{21}N_2O_4Cl$) C, H, N.

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