Expedited Articles

Diethylcarbamoylating/Nitroxylating Agents as Dual Action Inhibitors of Aldehyde Dehydrogenase: A Disulfiram−Cyanamide Merger

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Benzenesulfohydroxamic acid (Piloty's acid) was functionalized on the hydroxyl group with the *N*,*N*-diethylcarbamoyl group, and the hydroxylamine nitrogen was substituted with acetyl (**1a**), pivaloyl (**1b**), benzoyl (**1c**), and ethoxycarbonyl (**1d**) groups. Only compound **1d** inhibited yeast aldehyde dehydrogenase (AlDH) in vitro (IC_{50} 169 μM). When administered to rats, **1d** significantly raised blood acetaldehyde levels following ethanol challenge, thus serving as a diethylcarbamoylating/nitroxylating, dual action inhibitor of AlDH in vivo. A more potent dual action agent was *N*-(*N*,*N*-diethylcarbamoyl)-*O*-methylbenzenesulfohydroxamic acid (**5c**), which was postulated to release diethylcarbamoylnitroxyl (**9**), a highly potent diethylcarbamoylating/nitroxylating agent, following metabolic *O*-demethylation in vivo. The dual action inhibition of AlDH exhibited by **1d**, and especially **9**, constitutes a merger of the mechanism of action of the alcohol deterrent agents, disulfiram and cyanamide.

Introduction

Disulfiram (DSSD; tetraethylthiuram disulfide; Antabuse) has been used as an alcohol deterrent agent for nearly 50 years.[1] Its mode of action is known to be mediated by the inhibition of hepatic aldehyde dehydrogenase (AlDH; EC 1.2.1.3), the enzyme that converts ethanol-derived acetaldehyde (AcH) to acetate, resulting in the elevation of blood AcH levels on consumption of ethanol. This evokes a disulfiram−ethanol reaction (DER), a syndrome characterized by facial flushing, tachycardia, and a general feeling of malaise, ostensibly leading to alcohol avoidance.[2] A similar carbimide-ethanol reaction (CER) is elicited by cyanamide vis-à-vis its calcium salt, calcium carbimide,[3,4] an alcohol deterrent agent prescribed in Europe, Canada, and Japan. Both DSSD and cyanamide require metabolic bioactivation in vivo before AlDH inhibition is manifest. The active metabolite of DSSD has been isolated and shown to be methyl *N*,*N*-diethylthiolcarbamate *S*-oxide (DETC-MeSO; for structure, see Chart 2),[5] while the active inhibitor from cyanamide metabolism has been identified as nitroxyl (HN═O).[6]

Biliary excretion of *S*-(*N*,*N*-diethylcarbamoyl)glutathione after administration of DSSD to rats[7] suggests that DETC-MeSO can carbamoylate sulfhydryl groups, in particular, the SH group of the active site Cys-302 residue of AlDH, thus irreversibly inhibiting this enzyme. We have previously shown that nitroxyl can react with sulfhydryl reagents such as glutathione[8] and *N*-acetyl-L-cysteine (NAC)[9] to give the corresponding sulfinamides. Based on these studies, we postulated that the inhibition of AlDH by nitroxyl was a consequence of the nitroxylation of the active-site Cys-302 residue of the enzyme to an initial enzyme *N*-hydroxysulfenamide intermediate, followed by molecular rearrangement to an enzyme sulfinamide, resulting in irreversible inhibition.[10]

Earlier, we showed that chemical carbethoxylating

Chart 1

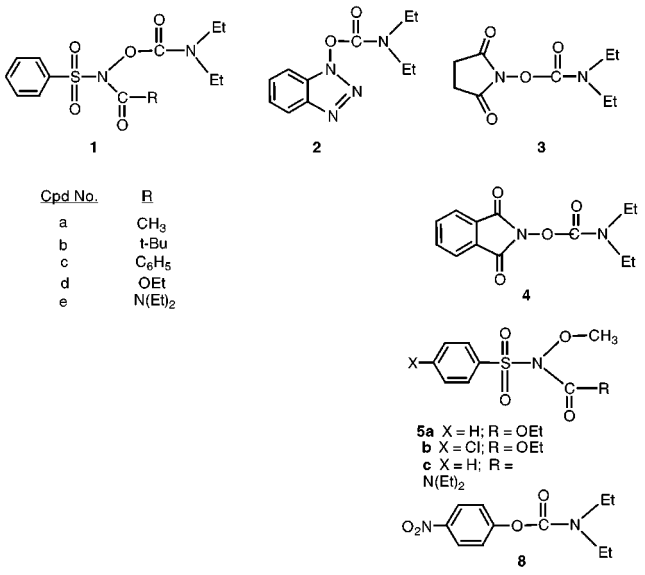
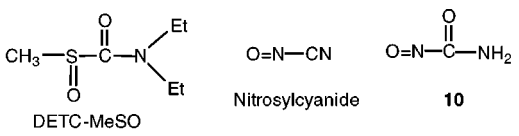


Chart 2



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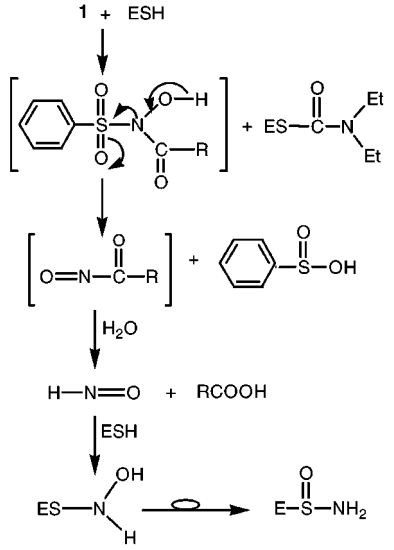
Scheme 1

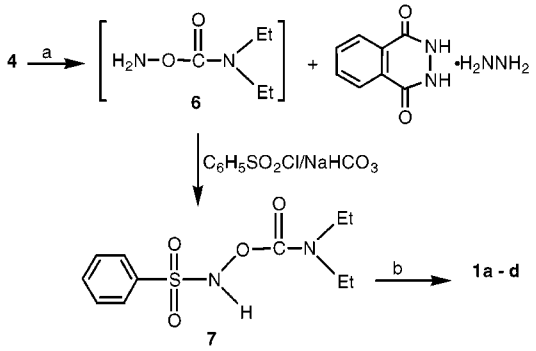


agents such as the *O*-ethoxycarbonylated derivatives of 1-hydroxybenzotriazole, *N*-hydroxyphthalimide, *N*-hydroxysuccinimide, and *N*-hydroxysaccharin were reasonably good inhibitors of yeast AlDH,[11] a readily available enzyme with a conserved Cys-302 and a sequence homology similar to the mammalian liver mitochondrial enzyme at the C-terminal active-site region.[12] We also demonstrated that prodrugs of nitroxyl that can also carbethoxylate the enzyme were powerful *dual action* inhibitors of yeast AlDH in vitro yet were totally inactive when tested in rats.[11,13] It was postulated that premature hydrolysis of the carbethoxy group, perhaps in plasma,[14] might be responsible for this lack of in vivo activity.

We now describe a series of compounds designed not only to diethylcarbamoylate the active-site Cys-302 of AlDH, thus mimicking the mechanism of action of DETC-MeSO, the active metabolite of DSSD,[5] but also to release nitroxyl, the putative inhibitor of AlDH produced by the catalase-mediated oxidation of the alcohol deterrent agent, cyanamide.[6]

Compounds **1a**−**e** (Chart 1) were designed to be such dual action inhibitors of AlDH, since, theoretically, they can diethylcarbamoylate the enzyme by initial esterase action intrinsic to this enzyme, followed by release of an equivalent of nitroxyl to nitroxylate any uncarbamoylated enzyme thiol at the active site (Scheme 1). The diethylcarbamoyl group being much less reactive than the carbethoxy group, extrahepatic reactions would be expected to be minimal. Compounds **2**−**4** are the *O*-diethylcarbamoyl analogues of the corresponding *O*-ethoxycarbonyl derivatives of 1-hydroxybenzotriazole, *N*-hydroxysuccinimide, and *N*-hydroxyphthalimide, respectively. The latter ethoxycarbonyl compounds were shown previously to inhibit yeast AlDH in vitro by carbethoxylating the enzyme.[11] Compound **5a** was intended to be the negative control for compound **1d** in vitro, since **5a** cannot nitroxylate in the absence of an *O*-demethylase (not found in AlDH) but can still carbethoxylate the enzyme based on the intrinsic reactivity of imides in general. Compound **5b**, which liberates nitroxyl following *O*-demethylation,[13] served as a positive control for the in vivo studies.

Scheme 2[a]



[a] (a) 3.3 H_2NNH_2, then H_2O_2; (b) Et_3N + Ac_2O (**1a**), *t*-BuCOCl (**1b**), C_6H_5COCl (**1c**), EtOCOCl (**1d**).

Results

Compounds **2**, **3**, and **4** were prepared by heating 1-hydroxybenzotriazole, *N*-hydroxysuccinimide, and *N*-hydroxyphthalimide, respectively, with excess diethylcarbamoyl chloride and triethylamine in THF under reflux for 4 h, while compound **5a** was readily prepared by carbethoxylating the sodium salt of *O*-methylbenzenesulfohydroxamic acid (*O*-methyl Piloty's acid) with ethyl chloroformate at room temperature.

The syntheses of compounds **1a**−**d** followed Scheme 2. The successful preparation of **6** required 3.3 equiv of hydrazine which necessitated destroying the excess with H_2O_2, while for the preparation of synthon **7**, a $NaHCO_3$ suspension was used as the acid scavenger, since use of triethylamine as base in the acylation reaction resulted in the formation of the disulfonylated product (structure not shown). However, all attempts to prepare *N*,*O*-bis(diethylcarbamoyl)benzenesulfohydroxamic acid (**1e**) from **7** under the usual conditions with diethylcarbamoyl chloride or with the reagent 4-nitrophenyl *N*,*N*-diethylcarbamate (**8**)[15] failed, even with heating under a variety of conditions, due to the extreme unreactivity of these carbamoylating reagents and the thermal instability of **7** (and **6**). Diethylcarbamoylation of **6** using **2** as well as the above reagents was also unsuccessful.

The inhibition of yeast AlDH by these diethylcarbamoyl compounds was examined in vitro by procedures described previously.[16] Except for **1d** (IC_{50} 169 μM), the remaining compounds of this series (Chart 1, except **6**), including compound **7**, did not inhibit this enzyme at a concentration of 0.5 mM (or 0.25 mM for **1c** due to aqueous insolubility). The inactivity of compounds **5a**,**b**, the negative controls for compound **1d** in this system, supports our conclusion that the inhibition of the enzyme by **1d** must be due to diethylcarbamoylation rather than to carbethoxylation (ethoxycarbonylation) of the active-site residue. It appears that the *N*-carbethoxy group in a mixed sulfonic/carboxylic imide structure as in **1d** and **5a,b** may not be as reactive as we had anticipated. The 4-chloro analogue **5b** was shown previously to inhibit rat liver mitochondrial AlDH in vivo following *O*-demethylation and the release of nitroxyl, since blood AcH levels of rats pretreated with **5b** were increased significantly over controls when subsequently challenged with ethanol.[13]

Compound **1d** was administered to rats as a suspension in 2% carboxymethylcellulose (CMC), and blood AcH levels were measured after ethanol challenge and

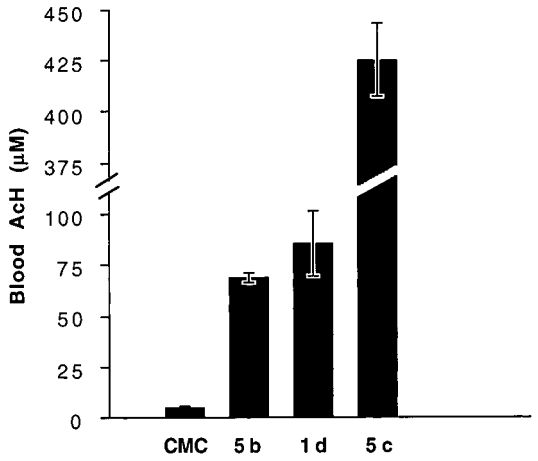


Figure 1. Blood AcH levels 1 h after ethanol administration in rats pretreated with compounds **1d** and **5c**. Compound **5b**, the positive control, is a known in vivo inhibitor of AlDH in rats. $N = 3$, except for **5b** where $N = 4$. There is no statistical difference between the AcH levels for **1d** and **5b**.

compared to that of **5b**, the positive control (Figure 1). It can be seen that **1d** was comparable to **5b** in raising ethanol-derived blood AcH in rats, a measure of the inhibition (>70%) of hepatic mitochondrial AlDH.[17]

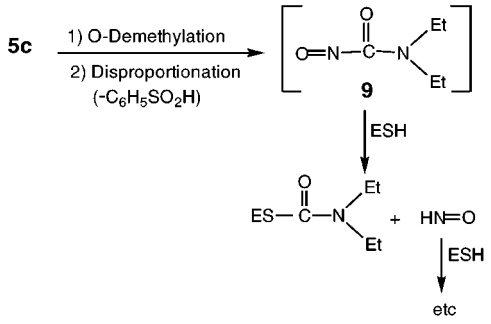
Discussion

The lack of inhibitory activity of compounds **2**−**4** against yeast AlDH confirmed our premise that the diethylcarbamoyl group is much less electrophilic than the ethoxycarbonyl group,[18] since the corresponding ethoxycarbonyl analogues of **2**−**4** were found to inhibit this enzyme, albeit with IC_{50}'s in the millimolar range.[11] The similar lack of activity of compounds **1a**−**c** against yeast AlDH was, however, surprising in view of the activity of prodrug **1d**, and we are unable to offer any explanation for this at this time. We considered the possibility that **1d** might be carbethoxylating rather than diethylcarbamoylating the enzyme. However, compounds **5a** as well as **5b** were inactive in this system, and we were unable to prepare the *N*,*O*-bis(diethylcarbamoyl) compound (**1e**) which might have resolved this question.[19] Nevertheless, the AlDH inhibitory activity of **1d** in rats fulfilled our goal of sufficiently attenuating the initial bioactivation step (Scheme 1) for the prodrug to be acted on by the *liver* enzyme and providing—for the first time—a diethylcarbamoylating/nitroxylating, dual action agent that inhibits AlDH in vivo.

DETC-MeSO has an activated diethylcarbamoyl group that readily reacts with glutathione and the active-site thiol of AlDH. The electrophilicity of this group increases significantly when the sulfoxide moiety is oxidized further to the sulfone, i.e., to DETC-$MeSO_2$.[20,21] However, it is clear from the present results that the diethylcarbamoyl group attached to the hydroxyl group of a sulfohydroxamic acid (compounds **1a**−**c**) or an *N*-hydroxyimide (compounds **3** and **4**) as carbamate esters, is not sufficiently electrophilic to react with the enzyme thiol. Thus, while **1d** did exhibit the desired properties that we had been seeking, a reevaluation of our structural design parameters and the hypothesis on which they were based appeared to be in order.

At this juncture, we sought a much more liver-specific functional group for the initial bioactivation step. We had shown earlier that such a liver-specific group indeed existed in the form of the *O*-methyl ether of sulfohydroxamic acid derivatives,[13,22] as exemplified here by compound **5b**. Accordingly, the *O*-methylated, *N*-diethylcarbamoylated derivative of Piloty's acid (compound **5c**) was prepared and tested in rats for its in vivo effect on AlDH.

Scheme 3



As shown in Figure 1, compound **5c** was highly effective in inhibiting the liver mitochondrial AlDH of rats, as reflected by the pronounced elevation in blood AcH levels following ethanol challenge. We envision the biochemical mechanism leading to this pharmacological end result to proceed as in Scheme 3. Specifically, the diethylcarbamoylnitroxyl intermediate **9**, produced following the hepatic cytochrome P-450-catalyzed *O*-demethylation of **5c** and subsequent elimination of benzenesulfinic acid, is now sufficiently active to diethylcarbamoylate the active-site enzyme thiol, thereby generating an equivalent quantity of nitroxyl to react further with any uninhibited enzyme. Although estimation of the relative degrees of carbamoylation versus nitroxylation of the enzyme by **5c** would be speculative, *dual action* is clearly indicated by the nearly 6-fold increase in activity compared to **5b**, a latent nitroxylating agent[13] (Figure 1).

It is noted that intermediate **9** bears a formal structural resemblance to DETC-MeSO (Chart 2) and also to nitrosyl cyanide (O═N−CN) produced in the oxidation of cyanamide by catalase/H_2O_2;[23] indeed, **9** closely resembles carbamoylnitroxyl (**10**; Chart 2) the partial hydrolysis product of nitrosyl cyanide.[23] Thus, the mechanism of inhibition of AlDH by disulfiram and the mode of action of cyanamide appears now to have merged in this postulated mechanism of inhibition of AlDH by diethylcarbamoylnitroxyl (**9**) generated from its prodrug **5c**.

Experimental Section

Chemistry. Melting points were taken on a Fisher-Johns hot-stage melting point apparatus and are uncorrected. IR spectra were recorded on a Nicollet FT-IR spectrophotometer. ^{1}H NMR spectra were recorded on a Varian Gemini 300-MHz NMR spectrometer. Chemical shifts (δ) are in parts per million (ppm) relative to $Si(CH_3)_4$, and coupling constants (J) are in hertz. Microanalyses were performed by M-H-W Laboratories, Phoenix, AZ. Thin-layer chromatography (TLC) was performed using Analtech silica gel GF Uniplates, and the products were visualized either by fluorescence quenching observed under UV light or by exposure to iodine vapor in an iodine chamber. Thick layer chromatography was performed using Analtech 1000-μm preadsorbent silica gel GF Uniplates. Organic solvents extracts were dried over Na_2SO_4 for 15−20 min before filtration.

Reagents and Chemicals. *N*-Hydroxyphthalimide, 1-hydroxysuccinimide, 1-hydroxybenzotriazole, benzenesulfonyl chloride, ethyl chloroformate, pivaloyl chloride, benzoyl chloride, *p*-(diethylamino)benzoic acid, thionyl chloride, diethylcarbamoyl chloride, and sodium hydride were purchased from Aldrich Chemical Co. (Milwaukee, WI). *N*-Carbethoxyl-*O*-methyl-4-chlorobenzenesulfohydroxamic acid (**5b**) was prepared as described.[14] Yeast AlDH, NAD^+, and glucose-6-phosphate dehydrogenase were purchased from Sigma Chemical Co. (St. Louis, MO).

General Procedure for the Preparation of 2−4. *O*-(Diethylcarbamoyl)-*N*-hydroxyphthalimide (4). *N*-Hydroxyphthalimide (48.9 g; 0.3 mol) was dissolved in 900 mL of THF, and the solution was cooled in an ice bath under an atmosphere of N_2. Et_3N (43.2 mL; 0.31 mol) was added over ca. 10 min (red color). Diethylcarbamoyl chloride (38.0 mL; 0.30 mol) was added dropwise over 30 min. The ice bath was then removed, and the reaction mixture was heated under reflux for 4 h (red color faded to yellow). The reaction mixture was allowed to cool to room temperature, EtOAc (500 mL) was added, and the mixture was washed twice with 1 N HCl (100 mL), twice with 1 N $NaHCO_3$ (100 mL, red color), and once with water (100 mL). After drying, the solvents were removed on a rotary evaporator in vacuo at 40 °C to give a colorless solid (60.9 g; 77.4% yield; mp 121.5−122 °C) which was used directly in the next reaction. An analytical sample, mp 126−127 °C, was obtained by recrystallization from EtOAc; R_f = 0.95 (EtOAc:hexane, 2/1). ^{1}H NMR: 7.74−7.88 (m, 4H, aryl-H), 3.48 (q, 2H, J = 6.9, CH_2N), 3.37 (q, 2H, J = 7.05, CH_2N), 1.331 (t, 3H, J = 6.81, CH_3), 1.198 (t, 3H, J = 6.96, CH_3). ^{13}C NMR: 150.823 (C=O carbamate), 161.615 (C=O imide). FTIR: (KBr) $\nu_{C=O}$ 1762; 1746 cm^{-1}. Anal. ($C_{13}H_{14}N2O_4$) C, H, N.

***O*-(Diethylcarbamoyl)-1-hydroxysuccinimide (3).** Prepared from 1-hydroxysuccinimide as described above for **4**. Colorless solid (57% yield); mp 109−111 °C (recrystallized from EtOAc−hexane); R_f = 0.27 (hexane:THF, 3:1). ^{1}H NMR: 3.40 (q, J = 8 Hz, 4H), 2.80 (s, 4H), 1.23 (t, J = 8 Hz, 6H). FTIR: (KBr) $\nu_{C=O}$ 1756, 1745 cm^{-1}. Anal. ($C_9H_{14}N_2O_4$) C, H, N.

***O*-(Diethylcarbamoyl)-1-hydroxybenzotriazole (2).** Prepared from 1-hydroxybenzotriazole as described above for **4**. Colorless solid (81.6% yield); mp 71−72 °C (recrystallized from EtOAc−hexane); R_f = 0.48 (hexane:THF, 3/1). ^{1}H NMR: 8.17−7.83 and 7.57−7.13 (2m, 4H), 3.52 (vbrm, 4H), 1.32 (vbrm, 6H). FTIR: (KBr) $\nu_{C=O}$ 1769 cm^{-1}. Anal. ($C_{11}H_{14}N_2O_4$) C,H,N.

***O*-(Diethylcarbamoyl)hydroxylamine (6). *O*-(Diethylcarbamoyl)benzenesulfohydroxamic Acid (7).** Compound **4** (2.62 g; 10 mmol) was dissolved in 100 mL of acetonitrile. The reaction vessel was flushed with N_2 for 5 min, and anhydrous N_2H_2 (3.3 equiv; 1.06 mL; 33 mmol) was added. *Caution: Anhydrous N_2H_2 should be stored and handled under N_2.* A white solid formed in a pale-yellow liquid suspension. The mixture was stirred at room temperature for 30 min and then allowed to stand in an ice bath for 3 h. TLC (toluene/EtOAc, 50:10) showed that the reaction was complete. The precipitate was collected and washed twice with 20 mL of ice-cold CH_3CN. [This precipitate, the hydrazine salt of phthalhydrazide, weighed 1.92 g (99% yield) after drying in vacuo.] The CH_3CN filtrate was cooled in an ice bath, and 30% w/w H_2O_2 (2.66 mL, 10.3 mmol) was added in four portions over 5 min when gas evolution ensued. After 1 h of stirring, at which point gas evolution had stopped and tests for H_2O_2 (iodide−starch paper) and N_2H_2 (salicylaldehyde/yellow fluorescence under long-wave UV light) were negative, $NaHCO_3$ (840 mg, 10 mmol) was added, followed by benzenesulfonyl chloride (1.28 mL, 10 mmol), and the reaction mixture was allowed to proceed at room temperature overnight. TLC at this time showed almost complete loss of **6** (R_f = 0.30; EtOAc/hexane, 1:2) and formation of **7** (R_f = 0.56; EtOAc/hexane, 1:2). The mixture was diluted with 5 volumes of saturated NaCl and extracted three times with ether, and the combined ether extracts were washed once with saturated NaCl, dried, and evaporated to dryness to give **7** (2.68 g, 98.5% yield) as a slightly yellow oil. Crystallization from toluene−hexane gave colorless crystals; mp 71.5−72 °C; R_f = 0.52 (toluene/EtOAc, 50:10). ^{1}H NMR: 7.52−7.96 (m, 5H, aryl H), 9.00 (s, 1H, NH), 3.22 (q, 2H, CH_2N, J = 7.14), 3.11 (q, 2H, CH_2N, J = 7.08), 1.11 (t, 3H, CH_3, J = 7.14), 0.88 (t, 3H, CH_3, J = 7.08). FTIR: $\nu_{C=O}$ 1727 cm^{-1}. Anal. ($C_{11}H_{16}N_2O_4S$) C,H,N,S.

General Procedure for the Preparation of Compounds 1a−d. *N*-Benzoyl-*O*-(diethylcarbamoyl)benzenesulfohydroxamic Acid (1c). Et_3N (408 μL; 2.93 mmol) was added to a solution of **7** (723 mg; 2.66 mmol) and benzoyl chloride (340 μL; 2.93 mmol) dissolved in 27 mL of CH_2Cl_2. The reaction mixture was stirred at room temperature for 24 h at which time TLC (toluene/EtOAc, 50:10) showed complete reaction of **7** (R_f = 0.52). The reaction mixture was evaporated to dryness and the residue was flash chromatographed using toluene/EtOAc (50:10) as eluant to give 700 mg (70% yield) of a colorless oil which crystallized on standing at 0 °C. Recrystallization from toluene−hexane gave **1c** as colorless crystals; mp 71−72 °C; R_f = 0.70 (toluene/EtOAc, 50:10). ^{1}H NMR: 7.35−8.11 (m, 10H, aryl H), 3.23 (brd q, 4H, J = 6.93), 1.04 (brd t, 6H, J = 6.93). FTIR: (KBr) $\nu_{C=O}$ 1763, 1697 cm^{-1}. Anal. ($C_{18}H_{20}N_2O_7S$) C,H,N,S.

***N*-Acetyl-*O*-(diethylcarbamoyl)benzenesulfohydroxamic Acid (1a).** This compound was prepared using acetic anhydride as described above for **1c.** Colorless solid; mp 93−94 °C (benzene-hexane); 58.4% yield; R_f = 0.57 (hexanes−EtOAc, 2:1). ^{1}H NMR: 7.52−8.05 (m, aryl-H, 5H), 3.36 (q, 4H, J = 7.11, $(CH_2)_2$), 2.12 (s, 3H, CH_3CO), 1.234 (t, 3H, J = 7.11, CH_3), 1.164 (t, 3H, J = 7.11, CH_3). FTIR: (KBr) $\nu_{C=O}$ 1708, 1768 cm^{-1}. Anal. ($C_{13}H_{18}N_2O_5S$) C,H,N,S.

***N*-Pivaloyl-*O*-(diethylcarbamoyl)benzenesulfohydroxamic Acid (1b).** Prepared as described above for **1c** except that pivaloyl chloride was used. Colorless solid; mp 110−111 °C (toluene−hexane); 60.1% yield; R_f = 0.56 (toluene−EtOAc, 50:5). ^{1}H NMR: 7.51−8.16 (m, 5H, aryl-H), 3.46 (dq, 4H, J = 7.20, 2(CH_2N)), 1.33 (t, 3H, J = 7.20, CH_3 carbamate), 1.23 (J = 7.31, t, 3H, CH_3 carbamate), 1.18 (s, 9H, $(CH_3)_3$. FTIR: (KBr) $\nu_{C=O}$ 1754, 1694 cm^{-1}. Anal. ($C_{14}H_{20}N_2O_7S$) C,H,N,S.

***N*-(Ethoxycarbonyl)-*O*-(diethylcarbamoyl)benzenesulfohydroxamic Acid (1d).** Prepared as described above for **1c** except that ethyl chloroformate was used. Colorless oil obtained by flash chromatography using toluene/EtOAc (50:10) as eluant; 83.2% yield; R_f = 0.68 (toluene/ETOAc, 50:10), R_f = 0.71 (toluene/EtOAc/Et_3N, 50:10:1), R_f = 0.55 (toluene/EtOAc/AcOH, 50:10:2). ^{1}H NMR: 7.52−8.10 (m, 5H, aryl H), 4.17 (q, 2H, J = 7.00, CH_2O), 3.37 (q, 4H, J = 6.95, CH_2N), 1.19 (t, 3H, J = 7.01, CH_3), 1.25 (brd t, 6H, $2CH_3$). ^{13}C NMR: 151.73 (C=O, carbamate), 149.513 (C=O, carbethoxy). FTIR: (KBr) $\nu_{C=O}$ 1759, 1759 cm^{-1}. λ_{max} (EtOH): 224.5 nm (ϵ 12 200). Anal. ($C_{14}H_{20}N_2O_6S$) C,H,N,S.

***N*-(Ethoxycarbonyl)-*O*-methylbenzenesulfohydroxamic Acid (5a).** Prepared from *O*-methylbenzenesulfohydroxamic acid[24] using the method described for **5b.**[13] This compound was purified by flash chromatography (silica gel 60) using toluene/EtOAc (50:10) as eluant (18.3% yield, colorless oil); R_f = 0.65 (toluene/EtOAc, 50:10). ^{1}H NMR: 7.506−8.011 (m, 5H, aryl H), 4.21 (q, J = 7.11, 2H, CH_2), 4.02 (s, 3H, OCH_3), 1.25 (t, J = 7.14, 3H, CH_3). FTIR: (KBr) $\nu_{C=O}$ 1751.6 cm^{-1}. Anal. ($C_{10}H_{13}NO_3S$) C,H,N,S.

***N*-(*N*,*N*-Diethylcarbamoyl)-*O*-methylbenzenesulfohydroxamic Acid (5c).** *O*-Methylbenzenesulfohydroxamic acid (1.87 g; 10 mmol) was dissolved in 10 mL of dry dimethylacetamide in an oven-dried three-necked 25-mL flask, equipped with a magnetic stirrer and a bubbler-guarded water-cooled condenser. A N_2 atmosphere was established in the flask. Under slow N_2 flow, solid NaH (368 mg; 15.3 mmol) was added all at once. The flask was resealed and stirred at room temperature for 2 h at which time the flask contained a cream-colored slurry. Diethylcarbamoyl chloride (1.94 mL; 15.3 mmol) was added, and the reaction mixture was warmed and stirred at 40 °C (water bath) for 19 h. The reaction mixture was then diluted with 100 mL of saturated NaCl and extracted with ether (4 × 16 mL). The combined ether extracts were washed with saturated NaCl, and the organic layer was dried and

evaporated to dryness in vacuo at room temperature to provide 3.09 g of a thick light yellow oil. This oil was flash chromatographed on silica gel 60 (160 g) using toluene as solvent. Fractions showing a single spot at $R_f = 0.32$ (toluene/EtOAc, 50:10) were combined and evaporated in vacuo at room temperature to give 1.88 g (66% yield) of **5c** as a clear, colorless oil; $R_f = 0.32$ (toluene/EtOAc, 50:10). ^{1}H NMR: 7.50−7.87 (m, 5H, aryl H), 3.30−3.80 (brd, 4H, CH_2N), 3.90 (s, 3H, CH_3O), 1.22 (t, 3H, CH_3, $J = 7.14$). FTIR: (KBr) $\nu_{C=O}$ 1706 cm^{-1}; λ_{max} (EtOH): 223 nm (ϵ 15 900). Anal. ($C_{12}H_{18}N_2O_4S$) C,H,N,S.

Inhibition of Yeast AlDH. The test compounds were evaluated for inhibitory activity against yeast AlDH as previously described.[16]

Pharmacological Evaluations in Vivo. These studies were performed in adherence with guidelines established in the *Guide for the Care and Use of Laboratory Animals* published by the U.S. Department of Health and Human Resources (NIH Publication 85-23, revised 1985). Animals were housed in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC), and the research protocol was approved by the subcommittee on Animal Studies of the Minneapolis VA Medical Center. This committee is vigorous in enforcing its charge of minimizing the use of animals in research.

Drug Administration Protocol. Sprague−Dawley male rats (Harlan Sprague−Dawley, Indianapolis, IN) weighing 165−180 g were fasted ∼24 h prior to the time of sacrifice. All drugs were finely ground and suspended in 2% carboxymethylcellulose (CMC). Doses of 1.0 mmol/kg were given ip as 1.0 mL/100 g of body weight. Ethanol was administered 1 h later and was given as a 20% (w/v) solution, 1.0 mL/100 g of body weight. The animals were sacrificed 1 h following ethanol administration, and blood was collected for AcH and ethanol measurements. Unlike the prodrugs of nitrosobenzene,[20] no evidence of toxicity was seen with these compounds (**1d, 5b,c**) over this short period.

Measurement of Blood AcH and Ethanol Levels. Blood AcH and ethanol levels were measured as previously described.[25]

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Supporting Information Available: Experimental procedures for the independent synthesis of **1d** from **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) For example, the *N*,*O*-bis(ethoxycarbonyl) derivative of 4-chlorobenzenesulfohydroxamic acid is much more readily hydrolyzed by human plasma than by porcine liver esterase (M. J. C. Lee and H. T. Nagasawa, unpublished).
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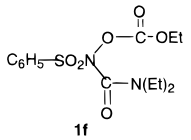
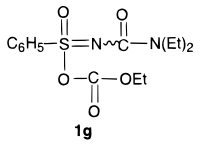
(18) For example, *N*,*N*-diethylcarbamoyl chloride is considerably less reactive than ethyl chloroformate.
(19) We also addressed the possibility that the positions of the ethoxycarbonyl and diethylcarbamoyl groups might be reversed as in **1f**. This is theoretically possible if **7** underwent an O-to-N acyl migration during the carbethoxylation step. However, **1d**, prepared unambiguously by carbethoxylation of **6** followed by benzenesulfonylation of the resulting product (structure not shown), had physiochemical properties which were indistinguishable from those of **1d** prepared via Scheme 2 (see Supporting Information). These data also exclude another possible isomeric structure for **1d**, viz., **1g**.



1f



1g

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