Latent Alkyl Isocyanates as Inhibitors of Aldehyde Dehydrogenase in Vivo

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On the basis of our previous observation that N^1-alkyl substituted chlorpropamide derivatives when administered to rats nonenzymatically eliminated *n*-propyl isocyanate, a known inhibitor of aldehyde dehydrogenase (AlDH), we have synthesized other latentiated *n*-propyl isocyanates as in vivo inhibitors of AlDH. N^1-Allylchlorpropamide **3** was, as expected, a potent inhibitor of hepatic AlDH in rats, as indicated by the 4-fold increase in the levels of ethanol-derived blood acetaldehyde relative to that elicited by chlorpropamide itself. Closely following in activity in decreasing order were N^3-(*n*-propylcarbamoyl)uracil (**7**), *N*-(*n*-propylcarbamoyl)saccharin (**6**), and the *S*-(*n*-propylcarbamoyl) derivative (**9**) of benzyl mercaptan. However, two hydantoin derivatives, **5** and **8**, were totally inactive in inhibiting AlDH in vivo. A prodrug of N^1-ethylchlorpropamide, *viz.*, its N^3-trifluoroacetyl derivative (**4b**), was a good in vivo inhibitor of AlDH, mimicking the activity of the parent N^1-ethylchlorpropamide. These results suggest that latent alkyl isocyanates are inhibitors of AlDH, giving further support to the hypothesis that the inhibition of AlDH in vivo by the hypoglycemic agent chlorpropamide may be due to the release of *n*-propyl isocyanate following metabolic bioactivation.

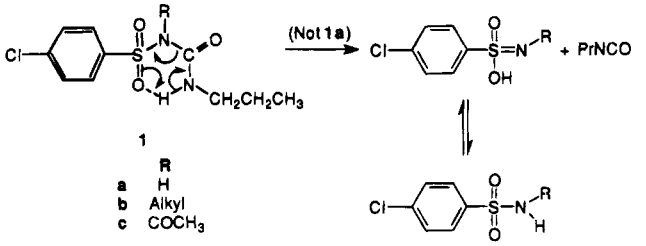
Approximately 30% of individuals treated with chlorpropamide (CP, **1a**), a sulfonylurea type oral hypoglycemic agent for the management of blood glucose, suffer an adverse reaction on consumption of ethanol[1] that mimics the disulfiram–ethanol reaction (DER).[2] This DER is manifested by facial flushing and a general feeling of malaise and is due to the inhibition of the hepatic enzyme aldehyde dehydrogenase (AlDH; EC 1.2.1.3) by a metabolite of CP[3] and consequent elevation of blood acetaldehyde (AcH).[4]

We had previously shown that chemical alkylation[5] or acetylation[6] on the N^1-position of CP gave rise to CP derivatives that showed the propensity to eliminate *n*-propyl isocyanate (PrNCO) nonenzymatically (Scheme 1). Indeed, **1c** could not be prepared because of this lability.[6] Since PrNCO is a good inhibitor of AlDH,[5] the DER elicited by CP may be due to the release of PrNCO in vivo after biofunctionalization of CP at this N^1-position. More recently, it was shown that the *S*-carbamoyl conjugates of PrNCO with cysteine (**2a**) and glutathione (**2b**) were potent inhibitors of AlDH in rats by virtue of the labile, reversible nature of the *S*-carbamoyl group of **2**, leading to the liberation of PrNCO in vivo (Scheme 2).[7]

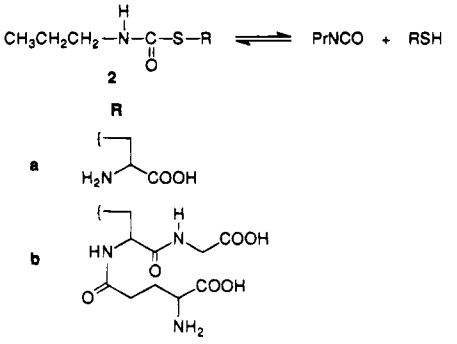
We postulated, therefore, that structurally analogous compounds with potential for releasing PrNCO, *i.e.*, *latentiated alkyl isocyanates*, might be good inhibitors of AlDH in vivo. Accordingly, a series of PrNCO prodrugs were synthesized and tested in rats for their ability to raise ethanol-derived blood AcH, a pharmacological end point that reflects the inhibition of hepatic AlDH by these compounds.[8]

Chart 1 shows the structures of the compounds (**3**–**9**) evaluated in this study. Compounds **4** and **5** were designed to be *pro*-prodrugs of PrNCO specifically targeted to the liver, whereby the acetyl or trifluoroacetyl group on **4** and the bridged two-carbon acetimide

Scheme 1



Scheme 2



bond in **5** must first be hydrolyzed by hepatic amidases or esterases before PrNCO could be released. Compound **6**, a saccharin derivative, is a functionally similar cyclic analog of compound **1c** and was expected to be a more stable mimic of the latter, whereas the thiocarbamate ester **9** is a simplified analog of **2a,b.**

Results and Discussion

N^1-Allyl-CP (**3**) was prepared by reacting allyl bromide with the sodium salt of CP (**1a**). Compound **4a** could not be prepared by acetylation of N^1-ethylchlorpropamide (N^1-EtCP, **1b**; R = Et) with acetic anhydride under a variety of conditions, the reaction taking the path for **1b** in Scheme 1 and leading ultimately to compound **12**. When reacted with ketene in the presence of triethylamine at room temperature, N^1-EtCP

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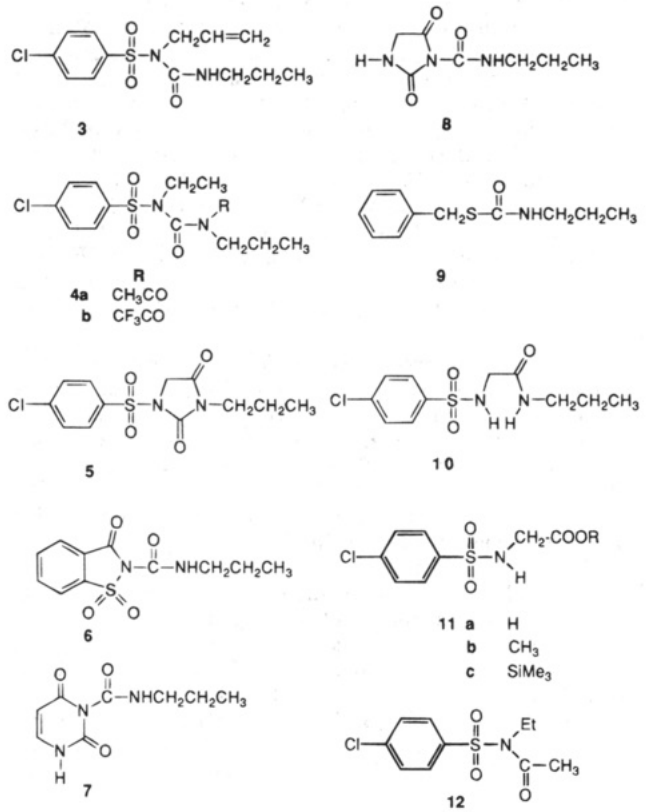
Chart 1



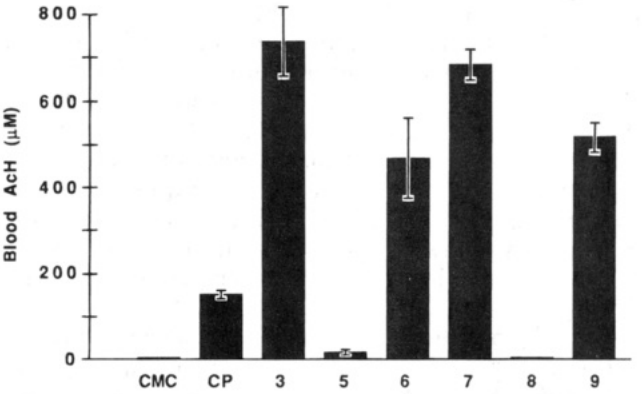


Figure 1. Elevation of blood AcH levels (±SEM) elicited by latent alkyl isocyanates following ethanol administration. Except for compounds **5** and **8**, $n = 6$, as explained under Drug Administration Protocol in the Experimental Section. For compounds **3**, **6**, **7**, and **9**, $P < 0.01$ vs CP control. Blood ethanol levels (not shown) for all groups were not statistically different from the levels shown by the vehicle (CMC) control group.

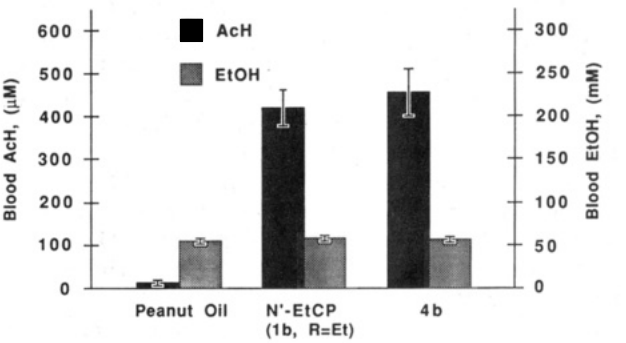


Figure 2. Elevation of ethanol-derived blood AcH elicited by the *pro*-prodrug **4b** compared to N^1-EtCP as reference standard. Peanut oil was used as drug administration vehicle. Other parameters were as in Figure 1. $P < 0.01$ for **4b** and N^1-EtCP vs peanut oil alone. There were no statistical differences between the blood ethanol levels of **4b**, N^1-EtCP, and the peanut oil control.

was recovered unchanged. However, **4b** was formed when N^1-EtCP was heated with neat trifluoroacetic anhydride. The (arylsulfonyl)hydantoin **5** formed spontaneously when *N*-[(4-chlorophenyl)sulfonyl]glycine methyl ester (**11b**), the corresponding trimethylsilyl ester **11c**, or the sodium salt of the acid (**11a**) was condensed with PrNCO. Compounds **6**–**9** were all readily prepared by the condensation of saccharin, uracil, hydantoin, and toluenethiol, respectively, with PrNCO.

The elevations in blood AcH elicited in rats by the intraperitioneal administration (1.0 mmol/kg) of compounds **3** and **5**–**9** [formulated in 2% carboxymethyl cellulose (CMC)] followed by ethanol administration (2.0 g/kg) 2 h later are compared to the results with CP as positive control in Figure 1. The most active compounds giving blood AcH levels 4 times that elicited by CP were the N^1-allyl derivative of CP (**3**) and N^3-(*n*-propylcarbamoyl)uracil (**7**). Closely following in inhibitory potency were the saccharin derivative **6** and the thiocarbamate ester **9**. That the observed elevation in ethanol-derived blood AcH elicited by N^1-allyl-CP (**3**) and, by analogy, compounds **6**, **7**, and **9** was due to the inhibition of the hepatic low-K_m AlDH (class II AlDH) by the released PrNCO, although not directly proved here, can be adduced from our previous studies. It was shown that concomitant to the elevation in blood AcH elicited by N^1-EtCP, a structural analog of **3** that releases PrNCO nonenzymatically, the hepatic mitochondrial AlDH was found to be inhibited 78%.[5b] Similarly, the N^1-methylated derivative of chlorpropamide inhibited the class II AlDH in intact rat liver mitochondria in vitro by 49% and osmotically disrupted mitochondria by 74% at a concentration of 1 mM.[5a]

The inactivity of compound **5**, which was anticipated to behave as a *pro*-prodrug of PrNCO, was unexpected. A possible metabolic path taken by compound **5** is to the ring-opened product **10**, thereby abrogating any possibility for PrNCO liberation. However, compound **10**, prepared by the amidation of the methyl ester **11b** with *n*-propylamine, was not the product of alkaline hydrolysis of **5**, this compound being **11a**, the expected product from the hydrolysis of the two-carbon bridge of **5** leading to PrNCO elimination. The lack of activity of compound **5** suggests that this bridged acetimide bond was not *enzymatically* hydrolyzed in vivo. On the other hand, compound **4b**, which was designed to be a prodrug of N^1-EtCP (**1b**; R = Et) and therefore a *pro*-prodrug of PrNCO, was a potent in vivo inhibitor of AlDH and equaled the activity of N^1-EtCP itself (Figure 2). Since the trifluoroacetyl group in **4b** was labile and solvolyzed in aqueous solution, **4b** was administered in peanut oil, as was N^1-EtCP which served as positive control. The total inactivity of the hydantoin derivative **8** may be rationalized on the basis that the *n*-propylcarbamoyl group on the N^3-position of **8** may have been hydrolyzed prematurely by enzymatic action, since N^3-acylhydantoins are metabolically labile to cleavage.[9]

Whereas N^1-acetyl-CP (**1c**), is unstable and spontaneously decomposed to *N*-acetyl-4-chlorobenzenesulfonamide and PrNCO on attempted preparation,[6] the functionally similar saccharin derivative **6** was readily isolated and characterized. This compound appears to be a good prodrug of PrNCO and inhibited hepatic AlDH

in vivo, as evidenced by the high levels of ethanol-derived blood AcH observed when administered to rats (Figure 1). Finally, the reversibility of the thiocarbamoyl group[10] of the thiocarbamate ester **9** is amply illustrated by its high inhibitory activity against AlDH in rats, suggesting the release of PrNCO in vivo.

In summary, latent alkyl isocyanates appear to be good inhibitors of hepatic AlDH by releasing an inhibitor of this enzyme, *viz.*, PrNCO, *e.g.*, from compounds **3, 4b**, **6**, **7,** and **9**, in vivo, thus lending credence to our hypothesis[5] that the inhibition of AlDH by chlorpropamide may be due to PrNCO release following bioactivation in vivo. The generally high reactivity of unlatentiated alkyl isocyanates toward biological macromolecules[11] precludes their use as drugs, since alkyl isocyanates are known to be active-site specific inhibitors of serum protease,[12] yeast alcohol dehydrogenase,[13] liver transglutaminase,[14] and elastase[15] by virtue of their action in carbamoylating the active-site −SH group of the enzymes. Hexyl isocyanate has also been shown to inhibit cholinesterase,[16] while the systemic and lethal toxicity of methyl isocyanate in humans is well known.[17] It is generally accepted that the active site of mammalian class II AlDH is Cys 302, the only conserved cysteine among the AlDHs whose sulfhydryl integrity, when compromised, leads to inhibition of the enzyme. For example, replacement of Cys 302 with Ala 302 by site-directed mutagenesis leads to an inactive enzyme.[18] Thus, it is likely that the PrNCO released from these latent isocyanates is carbamoylating this active-site cysteine moiety on AlDH.

Latentiated alkyl isocyanates that are selectively targeted to the specific tissue enzymes need to be developed before clinically useful drugs can ensue. For example, selectively targeted, latent isocyanates that inhibit human leukocyte elastase have been suggested for potential use in the treatment for emphysema.[19] Whether the PrNCO prodrugs described here will be useful as alcohol deterrent agents or applicable in another capacity will depend on their specificity. We have not evaluated other pharmacological profiles of the compounds listed in Chart 1, but active members of this series, *viz.*, compounds **3, 6**, **7**, and **9**, caused skeletal muscle relaxation in rats treated acutely with these agents in a manner similar to that previously observed for the N^1-alkylated chlorpropamide derivatives.[5] It is noteworthy that the *pro*-prodrug form **4b** did not elicit this pharmacological response.

Experimental Section

Organic chemicals were purchased from Aldrich Chemical Co., Milwaukee, WI. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, or M-H-W Laboratories, Phoenix, AZ. Infrared spectra were taken on a Digilab FTS-40 infrared spectrophotometer. EI-MS were recorded on a Kratos MS 25 mass spectrometer. ^{1}H-NMR spectra were recorded on a Varian T-60A (60 MHz), a Bruker AC-200 (200 MHz) or a Nicolet NT-300WB (300 MHz) spectrophotometer. For the 60 MHz spectra, Silanor C [$CDCl_3$ containing 1% tetramethylsilane (TMS) as a reference] was used as solvent, whereas for the 200 and 300 MHz spectra, $CDCl_3$ was used both as solvent and reference unless otherwise indicated.

1-(3-Propenyl)-1-[(4-chlorophenyl)sulfonyl]-3-*n*-propylurea (3). NaH (50% in mineral oil, 0.50 g, 10 mmol) was added to **1a** (2.77 g, 10 mmol) in dimethylacetamide (20 mL), and the mixture was stirred for 20 min. Allyl bromide (1.40 g, 11.5 mmol) was then added, and stirring was continued for 20 h. The suspension was then diluted with H_2O (50 mL), and the mixture was extracted with Et_2O (5 × 20 mL). Evaporation of the Et_2O extract gave an oily residue that was purified by flash chromatography on a silica gel column using hexane:THF (10:1) as eluent. Fractions containing pure **3** were combined, and the solvent was evaporated to give 2.24 g of **3** (71% yield). IR (neat, cm^{-1}): 3404 (NH), 1701 (C=O), 1527 (NH deform), 1355, 1160 (SO_2N). NMR ($CDCl_3$, 300 MHz): δ 7.64 (A_2B_2 q, J = 8.7 Hz, $\Delta\nu$ = 80 Hz, 4 H, ArH), 7.16 (s, 1 H, NH), 5.82 (m, 1 H, C*H*=CH_2), 5.26 (m, 1 H, *trans*-CH=C*H*H), 5.16 (m, 1 H, *cis*-CH=C*H*H), 4.27 (d, J = 6.0 Hz, NCH_2CH), 3.22 (m, 2 H, C$H_2$$CH_2CH_3$), 1.54 (m, 2 H, $CH_2CH_2CH_2CH_3$), 0.92 (t, J = 7.4 Hz, 3 H, $CH_2CH_2CH_2CH_3$). Anal. ($C_{13}H_{17}N_2O_3SCl$) C, H, N.

1-Ethyl-1-[(4-chlorophenyl)sulfonyl]-3-*n*-propyl-3-(trifluoroacetyl)urea (4b). A solution of **1b** (R = Et; 0.40 g, 1.3 mmol) and trifluoroacetic anhydride (3 mL) was placed into each of two pressure tubes (5 mL). The tubes were capped and heated at 70 °C for 66 h, and the combined contents (yellow) were concentrated on a rotary evaporator. The residue was subjected to the action of a vacuum pump to give 1.07 g of a yellow liquid. Crystallization from ethyl acetate−hexane (seeded with crystals from a previous reaction) gave 0.32 g, mp 53−54 °C (crop 1), and 0.48 g, mp 53−54 °C (crop 2). Third and fourth crops totaled 0.10 g, mp 52−53 °C. Total yield: 0.90 g (86%). IR (KBr, cm^{-1}): 1729, 1714 (C=O's), 1584, 1477. EI-MS: (*m/z*) 336, 209, 175. NMR ($CDCl_3$, 300 MHz): δ 7.72 (A_2B_2 q, J = 8.4 Hz, $\Delta\nu$ = 100 Hz, 4 H, ArH), 3.75 (q, J = 7.0 Hz, 2 H, C$H_2$$CH_3$), 3.61 (t, J = 7.8 Hz, 2 H, C$H_2$$CH_2$-$CH_3$), 1.67 (m, 2 H, CH_2C$H_2$$CH_3$), 1.30 (t, J = 7.0 Hz, 2 H, CH_2CH_3), 0.90 (t, J = 7.4 Hz, 3 H, CH_2CH_2CH_3). Anal. ($C_{14}H_{16}ClF_3N_2O_4S$) C, H, N.

***N*^{1}-[(4-Chlorophenyl)sulfonyl]-*N*^{3}-*n*-propylimidazolidine-2,4-dione (5).** Mixtures of *N*-[(4-chlorophenyl)sulfonyl]-glycine methyl ester (**11a**; 1.32 g, 5.0 mmol), triethylamine (0.80 g, 7.5 mmol), and *n*-propyl isocyanate (2.12 g, 25 mmol) in each of three pressure tubes (5 mL) were heated at 80 °C. When the solids had nearly dissolved, the contents suddenly solidified and filled the tubes. The excess reagent was hydrolyzed by stirring in 1 N HCl (100 mL) and the product extracted into EtOAc (required warming). After drying (Na_2SO_4), the solvent was evaporated to dryness and the residue recrystallized from CH_2Cl_2−hexane in two crops (3.46 g, 66.5% yield), colorless needles, mp 180−181 °C. IR (KBr, cm^{-1}): 1800, 1732 (C=O's), 1367, 1168 (SO_2N). NMR ($CDCl_3$ + 1 drop of DMSO-d_6, 300 MHz): δ 7.79 (A_2B_2 q, J = 8.6 Hz, $\Delta\nu$ = 136 Hz, 4 H, ArH), 4.33 (s, 2 H, NCH_2CO), 3.42 (t, J = 7.4 Hz, 2 H, C$H_2$$CH_2CH_3$), 1.61 (m, 2 H, CH_2C$H_2$$CH_3$), 0.87 (t, J = 7.4 Hz, 3 H, CH_2CH_2CH_3). Anal. ($C_{12}H_{13}N_2O_4SCl$) C, H, N.

***N*-(*n*-Propylcarbamoyl)saccharin (6).** A mixture of saccharin (0.50 g, 2.7 mmol), *n*-propyl isocyanate (0.91 g, 11 mmol), and acetone (3 mL) was placed in a pressure tube and heated for 1 h at 75 °C. After cooling, the product was collected and recrystallized from THF−hexane to give 0.46 g of **6** (63% yield): mp >170 °C dec (releasing lachymator) to saccharin. IR (KBr, cm^{-1}): 3350 (NH), 1747, 1710 (C=O's), 1532 (NH deform), 1361, 1193 (SO_2). NMR ($CDCl_3$, 300 MHz): δ 8.80 (m, 4 H, ArH), 3.40 (m, 2 H, C$H_2$$CH_2CH_3$), 1.67 (m, 2 H, CH_2C$H_2$$CH_3$), 0.99 (t, J = 7.5 Hz, 3 H, CH_2CH_2CH_3). Anal. ($C_{11}H_{12}N_2O_4S$) C, H, N.

***N*^{3}-(*n*-Propylcarbamoyl)uracil (7).** A mixture of uracil (1.12 g, 10 mmol), triethylamine (5 mL, 3.63 g, 36 mmol), and *n*-propyl isocyanate (4.54 g, 53 mmol) in acetone (50 mL) was stirred and heated under reflux for 18 h. The cooled reaction mixture was then filtered, and the filtrate was evaporated to 20 mL. The workup followed the procedure for **5.** The product that precipitated when the EtOAc volume was reduced was collected and washed with Et_2O to give colorless plates of **7**, 0.57 g (29% yield), mp >160 °C dec with sublimation (releasing a lachrymator) to uracil. IR (KBr, cm^{-1}): 3292 (NH), 1800, 1750 (sh), 1742, 1726 (sh) (C=O's), 1536 (NH deform). NMR ($CDCl_3$, 300 MHz): δ 9.10 (1 H, NH), 8.90 (1 H, NH), 8.43 (d, J = 8.6 Hz, 1 H, NHC*H*=CH), 5.90 (d, J = 8.6 Hz, 1 H, COC*H*=CH), 3.37 (m, 2 H, C$H_2$$CH_2CH_3$), 1.64 (m, 2 H,

$CH_2CH_2CH_3$), 0.98 (t, J = 7.4 Hz, 3 H, $CH_2CH_2CH_3$). Anal. ($C_8H_{11}N_3O_3$) C, H, N.

N^3-(n-Propylcarbamoyl)imidazolidine-2,4-dione (8). A mixture of hydantoin (6.00 g, 60 mmol), triethylamine (10.9 g, 110 mmol), n-propyl isocyanate (4.54 g, 53.3 mmol), and acetone (150 mL) was heated under reflux for 20 h. After workup as for **7** above, the product was recrystallized from EtOAc–hexane to give **8**, 3.50 g (31% yield), mp 157–158 °C; IR (KBr, cm^{-1}): 3327 (NH), 1755, 1731, 1658 (C=O's), 1563 (NH deform). NMR ($CDCl_3$, 300 MHz): δ 8.16 (s, 1 H, NH), 7.68 (s, 1 H, NH), 4.37 (s, 2 H, $NHCH_2CO$), 3.29 (m, 2 H, $CH_2CH_2CH_3$), 1.58 (m, 2 H, $CH_2CH_2CH_3$), 0.96 (t, J = 7.6 Hz, 3 H, $CH_2CH_2CH_3$). Anal. ($C_7H_{11}N_3O_3$) C, H, N.

S-Benzyl-n-propylthiocarbamate (9). To a solution of benzyl mercaptan (5.29 g, 42.6 mmol) in THF (25 mL) was added n-propyl isocyanate (7.26 g, 85.4 mmol). After heating under reflux for 8 h, the solvent was evaporated to give crude **9** as an oil. Flash chromatography (silica gel, 35–70 mesh) using CH_2Cl_2 as eluent gave 7.51 g of **9** (84% yield), mp 66.5–68.5 °C. IR (KBr, cm^{-1}): 3271 (NH), 1636 (C=O), 1584 (NH deform). NMR ($CDCl_3$, 300 MHz): δ 7.28 (m, 5 H, ArH), 5.30 (s, 1 H, NH), 4.16 (s, 2 H, $ArCH_2S$), 3.27 (m, 2 H, $CH_2CH_2CH_3$), 1.54 (m, 2 H, $CH_2CH_2CH_3$), 0.92 (t, J = 7.5 Hz, 3 H, $CH_2CH_2CH_3$). Anal. ($C_{11}H_{15}NO_5$) C, H, N.

N-[(4-Chlorophenyl)sulfonyl]glycine N-Propylamide (10). Compound **11b** (0.79 g, 3.0 mmol) and n-propylamine (3.0 mL) were placed in a pressure tube and heated for 1 h at 100 °C. After cooling, the mixture was dissolved in THF and decolorized with charcoal and the solvent evaporated to dryness. The product was recrystallized from THF–hexane to give **10**, 0.63 g (72% yield), mp 148–149 °C. IR (KBr, cm^{-1}): 3296, 3264 (NH), 1659 (C=O), 1587 (NH deform), 1321, 1156 (SO_2N). NMR ($CDCl_3$, 300 MHz): δ 7.66 (A_2B_2 q, J = 8.4 Hz, $\Delta\nu$ = 87.5 Hz, 4 H, ArH), 6.07 (s, 1 H, NH), 5.35 (s, 1 H, NH), 3.58 (d, J = 5.7 Hz, 2 H, $NHCH_2CO$), 3.20 (m, 2 H, $CH_2CH_2CH_3$), 1.49 (m, 2 H, $CH_2CH_2CH_3$), 0.90 (t, J = 7.4 Hz, 3 H, $CH_2CH_2CH_3$). Anal. ($C_{11}H_{15}N_2O_3SCl$) C, H, N.

N-[(4-Chlorophenyl)sulfonyl]glycine (11a). To a cooled (ice bath), stirred solution of **11b** (2.40 g, 10.1 mmol) in acetone (25 mL) and methanol (12.5 mL) was added, dropwise with stirring, 4 N NaOH (7.5 mL) while maintaining the temperature at <5 °C. The solution was then concentrated to 10 mL, diluted with H_2O (30 mL), and acidified with HCl. After extraction with EtOAc, the extract was dried (Na_2SO_4) and concentrated to 25 mL. Addition of hexane to the boiling solution (15 mL) resulted in the precipitation of the product which was collected, 1.99 g (80% yield), mp 180–181 °C, sublimation >150 °C. IR (KBr, cm^{-1}): 3352 (NH), 1712 (C=O), 1324, 1154 (SO_2N). NMR ($CDCl_3$, 300 MHz): δ 7.65 (A_2B_2 q, J = 8.4 Hz, $\Delta\nu$ = 104 Hz, 4 H, ArH), 6.01 (m, 1 H, NH), 3.72 (d, J = 5.1 Hz, 2 H, $NHCH_2CO$). Anal. ($C_8H_{18}NO_4SCl$) C, H, N.

N-[(4-Chlorophenyl)sulfonyl]glycine Methyl Ester (11b). To a cooled (ice bath), stirred mixture of glycine methyl ester hydrochloride (10.0 g, 80 mmol) and triethylamine (18.2 g, 180 mmol) in $CHCl_3$ (150 mL) was added, dropwise, a solution of 4-chlorobenzenesulfonyl chloride (15.0 g, 71 mmol) in chloroform (50 mL). The ice bath was removed and the mixture allowed to stand overnight at room temperature. After washing with 3 N HCl (3 × 80 mL) and H_2O (3 × 50 mL), the $CHCl_3$ was evaporated to give a solid residue which was recrystallized from THF–hexane to give colorless crystals of **11b**, 17.2 g (92% yield), mp 135–136 °C. NMR ($CDCl_3$, 300 MHz): δ 7.79 (A_2B_2 q, J = 8.4 Hz, $\Delta\nu$ = 93.8 Hz, 4 H, ArH), 5.13 (s, 1 H, NH), 3.81 (d, J = 4.6 Hz, 2 H, $NHCH_2CO$), 3.66 (s, 3 H, OCH_3). Anal. ($C_9H_{10}NO_4SCl$) C, H, N.

Attempted Acetylation of 1-Ethyl-1-[(4-chlorophenyl)-sulfonyl]-3-n-propylurea (1a; R = Et). Mixtures of **1a** (R = Et) (1.00 g, 33 mmol), triethylamine (0.50 mL, 36 mmol), and acetic anhydride (3.0 mL, 27 mmol) in each of three pressure tubes (5 mL) were heated at 130 °C for 20 h. After cooling, the contents were poured into 2 N NaOH (100 mL) and stirred for 1 h. The mixture was extracted with EtOAc, and the extract was decolorized (charcoal), dried (Na_2SO_4), and concentrated to dryness. The yellow solid was recrystallized from ether–n-hexane (decolorized with charcoal) to give colorless, chunky crystals of N-acetyl-N-ethyl-4-chlorobenzenesulfonamide (**12**), 1.37 g (40% yield), mp 88–89 °C. IR (KBr, cm^{-1}): 1685 (C=O), 1351, 1172 (SO_2N). Anal. ($C_{10}H_{12}NO_3SCl$) C, H, N.

Pharmacological Evaluation. 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.

Drug Administration Protocol. Except for **4b** and N^1-EtCP where peanut oil was the vehicle, the test compounds were suspended in 2% aqueous carboxymethyl cellulose and administered at a dose of 1.0 mmol/kg, ip, at zero time to fasted male rats of Sprague–Dawley descent (Harlan Sprague–Dawley, Inc., Indianapolis, IN) weighing 160–200 g. Ethanol (2.0 g/kg, ip) was given at 2 h, and the animals were sacrificed 1 h following the ethanol dose for measurement of blood acetaldehyde. chlorpropamide-treated animals served as positive control, and CMC was used as the vehicle control. Three animals were used per group, and when in vivo inhibition of AlDH was indicated, the experiment was repeated with another group of three animals. However, when the initial experiment showed no activity for the compound, the experiment was *not* repeated in compliance with the mandate of our Subcommittee on Animal Studies to keep animal usage to a minimum.

Blood Acetaldehyde Determination. Blood AcH levels were measured 1 h after the administration of ethanol in treated and control animals as previously described.[8]

Statistical Analysis. Experimental values are expressed as means ± SEM. Statistical significance was determined using one-way analysis of variance (ANOVA). Where significance was indicated, the Neuman–Keuls criteria were used to compare the means of multiple groups. Statistical significance is indicated by P values of ≤ 0.05.

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