

N¹-Hydroxylated Derivatives of Chlorpropamide and Its Analogs as Inhibitors of Aldehyde Dehydrogenase in Vivo

Melinda J. C. Lee,[†] James A. Elberling, and Herbert T. Nagasawa*

Department of Medicinal Chemistry, University of Minnesota, and Medical Research Laboratories, VA Medical Center, Minneapolis, Minnesota 55417

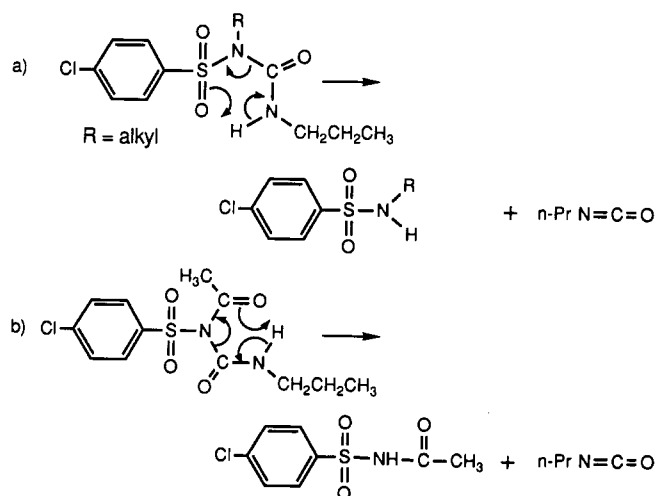
Received December 20, 1991

Certain (arylsulfonyl)urea hypoglycemic drugs exemplified by chlorpropamide (CP) are known to interact pharmacologically with alcohol (ethanol) to elicit a chlorpropamide-alcohol flushing (CPAF) reaction that is reminiscent of the disulfiram-ethanol reaction (DER). In the present structure-activity study, designed to elucidate the mechanism of inhibition of aldehyde dehydrogenase (AIDH) by CP, we discovered that the N¹-methoxy derivative of CP **2a** was a potent inhibitor of AIDH in vivo similar in activity to that of the N¹-ethyl derivative **2b**. Both **2a** and **2b** can release *n*-propyl isocyanate, a known inhibitor of AIDH, nonenzymatically. However, (arylsulfonyl)carbamates that are structurally analogous to **2a** were also active inhibitors of AIDH, whereas the corresponding (arylsulfonyl)carbamate analogs of **2b** were uniformly without activity. We propose a mechanism of bioactivation of **2a** and its analogs that involves initial O-demethylation followed by disproportionation and solvolysis of the intermediate formed to release nitroxyl, the putative inhibitor of AIDH.

Chlorpropamide (CP, **1**), a sulfonylurea-type oral hypoglycemic agent, when taken in combination with alcoholic beverages even in moderate amounts, elicits an adverse physiological reaction manifested visibly by facial flushing in approximately 30% of individuals who use this drug to control blood sugar.¹ This chlorpropamide-alcohol flushing (CPAF) reaction is reminiscent of the disulfiram-ethanol reaction² (DER) and is caused by the inhibition of hepatic as well as erythrocyte aldehyde dehydrogenase (EC 1.2.1.3; AIDH), leading to the elevation of blood acetaldehyde (AcH) following ingestion of ethanol.³ Since CP itself does not inhibit AIDH in vitro, we,⁴ and others,⁵ have postulated that it must undergo bioactivation in vivo to a reactive metabolite that inhibits this enzyme.

We had previously established that oxidative biotransformation of the *n*-propyl side chain of CP does not produce any metabolic species that inhibit AIDH.⁴ However, based on chemical evidence that *alkylation* or *acetylation* of the N¹-position of CP led to products that displayed the propensity to eliminate the *n*-propyl side chain nonenzymatically as *n*-propyl isocyanate (Scheme I), the latter

Scheme I



a potent inhibitor of AIDH, we postulated that biological functionalization of CP at this N¹-position must be involved in its bioactivation mechanism.⁶ In support of this, it was shown that N³-methyl-CP (**3**), where the N³-H is substituted with methyl group, or CP analogs, where O replaced this N³-H bridge such that isocyanate elimination was blocked or made impossible, were devoid of any biological activity in vivo. Moreover, N¹-tert-butyl-CP (**4**) was found to be unstable at physiological pH and temperature, giving rise spontaneously to *N*-tert-butyl-4-chlorobenzenesulfonamide and, by deduction, *n*-propyl isocyanate.⁶ Thus, these N¹-substituted analogs of CP (Scheme I) may be construed to represent *prodrugs* of *n*-propyl isocyanate that can release this enzyme inhibitor nonenzymatically in vivo.

Indeed, we have shown recently that *S*-(*n*-propylcarbamoyl)-L-cysteine and *S*-(*n*-propylcarbamoyl)-L-glu-

[†] Present address: Chemistry Department, St. Cloud State University, St. Cloud, MN 56301.

(1) (a) Anonymous. Alcohol sensitivity to sulphonylureas. *Br. Med. J.* 1964, 2, 586-587. (b) Leslie, R. G. D.; Pyke, D. A. Chlorpropamide-alcohol flushing: a dominantly inherited trait associated with diabetes. *Brit. Med. J.* 1978, 2, 1519-1521.

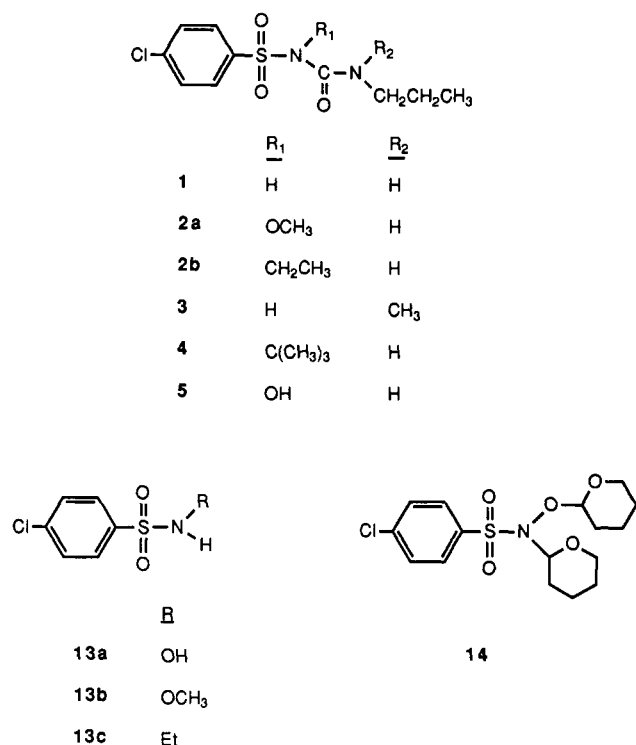
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tathione, which are the S conjugates of *n*-propyl isocyanate with L-cysteine and glutathione, respectively, also function as prodrugs of *n*-propyl isocyanate and inhibit AIDH in rats by virtue of their chemical reversibility in vivo, raising ethanol-derived blood AcH several-fold higher than elicited by CP itself.⁷

In search of other biologically relevant substituents at this N¹-position of CP, we considered the possibility of the hydroxyl group even though we are unaware of any biological N-hydroxylation reactions on the sulfonamide nitrogen of sulfonamides or on sulfonylureas. We were unable to prepare N¹-hydroxy-CP (5) for pharmacological evaluation (vide infra), but did succeed in preparing the corresponding oxygen-masked N¹-methoxy-CP (2a). This latter CP analog turned out to be a highly potent in vivo inhibitor of AIDH in rats, as reflected by blood AcH levels after ethanol challenge of four times that seen with CP itself (Figure 1). However, since 2a can be considered to be an isostere of N¹-ethyl-CP (2b) and could also release *n*-propyl isocyanate in vivo by the same mechanism depicted in Scheme Ia, other N¹-OMe analogs of CP were prepared, and systematic modifications were made not only in the side chain, but also in the functionality of the molecule, e.g., from a sulfonylurea to a sulfonylcarbamate (Chart I). We then conducted a detailed structure-activity study comparing the N¹-OMe with the N¹-Et-substituted sulfonylureas and the N-OMe with the N-Et-substituted sulfonylcarbamates. The results of these experiments to be described herein suggest that the mechanism of action of these N¹-OMe analogs of CP may be completely different from that depicted in Scheme I, viz., may, in fact, involve the release of nitroxyl (nitrosyl hydride, HN=O) instead of isocyanate as the putative inhibitor of AIDH.

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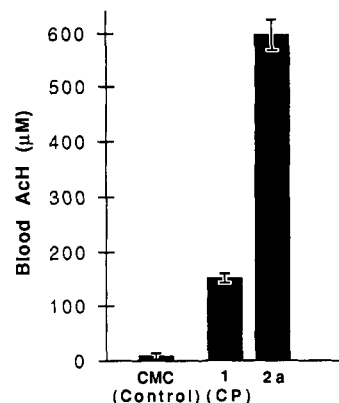
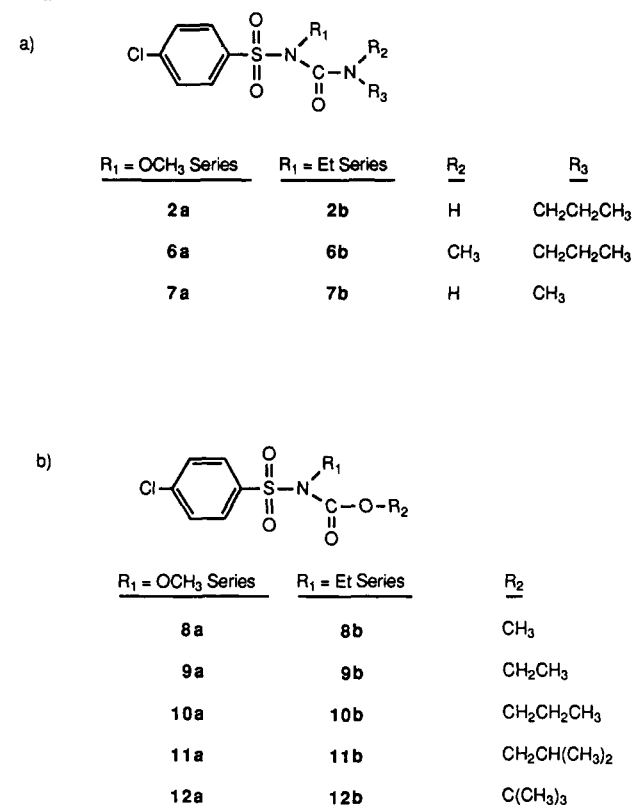


Figure 1. Effect of the N¹-methoxy derivative of chlorpropamide (2a) on ethanol-derived blood AcH in rats. The drug administration protocols and blood AcH assay methods are described in the Experimental Section. The results are given as mean $\pm$ SEM from duplicate determinations of blood AcH from three or more animals.

Chart I

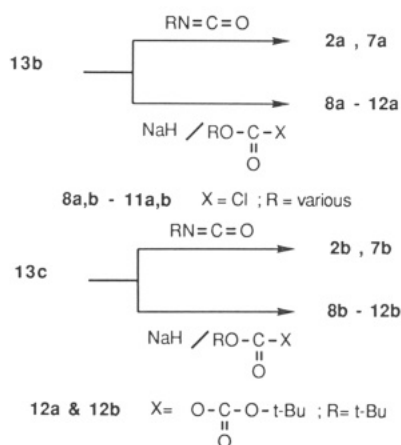


Chemistry

In efforts to prepare N¹-hydroxy-CP (5), we first sought to protect the hydroxyl function of the synthon 4-chloro-N-hydroxybenzenesulfonamide (13a) by selective O-acetylation in the manner described for N-hydroxybenzenesulfonamide.⁸ However, the N,O-bis-acetyl compound was formed from 13a under a variety of acetylation conditions. Use of pyruvonnitrile⁹ also did not result in selective O-acetylation. Attempted O-dihydropyranylation of 13a also produced what we believe to be the N,O-bis-dihy-

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Scheme II



dropyran derivative 14. For these reasons, we elected to proceed with our studies using the *O*-methyl derivative 13b as synthon since the methyl group can theoretically be removed in vivo by enzymatic *O*-demethylation. Sulfonylation of methoxylamine (*O*-methylhydroxylamine) with 4-chlorobenzenesulfonyl chloride gave 13b which was then condensed with *n*-propyl isocyanate to give the first of our target compounds 2a. Various other carbamoylating and carboalkoxylating agents were also reacted with 13b to give the corresponding compounds in the *N*¹-OMe series and with 13c to give the compounds in the *N*¹-Et series (Chart I and Scheme II). For the preparation of 6a and 6b, *N*-methyl-*N*-propylcarbamoyl chloride was coupled with 13b and 13c, respectively. These reactions all proceeded without difficulty, and the target compounds were readily characterized by their IR and NMR spectra, and by elemental analyses.

Biological Results and Discussion

As alluded to earlier, the *N*¹-OMe derivative 2a of CP was found to be a potent in vivo inhibitor of AIDH (Figure 1). Its structural similarity to the corresponding *N*¹-Et derivative 2b suggests that the mechanism depicted in Scheme Ia may also be operative here. However, compound 6a with an *N*¹-OMe substituent and where the *N*³-H has been substituted with a methyl group was nearly as active as 2a in elevating ethanol-derived blood AcH in rats, whereas the corresponding *N*¹-Et compound (6b) was much less active (Figure 2). Similarly, 7a, where the *N*¹-substituent is OMe and *N*³ is substituted with CH₃ and H, was highly active in vivo, whereas the *N*¹-Et analog 7b had less activity. It is recalled that chlormethamide (CH₃ replaces *n*-propyl at *N*³ in CP, structure not shown) was devoid of activity,⁴ whereas *N*¹-substitution with the ethyl group to give compound 7b restored the activity (Figure 2). Here again, if the mechanism of bioactivation were the same for 6a and 6b, or for 7a and 7b, the a and b series should have shown equivalent activity, just as equivalent activity was observed for 2a and 2b.

More compelling evidence that the active species from the *N*¹-OMe sulfonylureas must be different from that depicted for the *N*¹-Et sulfonylureas in Scheme Ia can be adduced from the results of the sulfonylcarbamate series

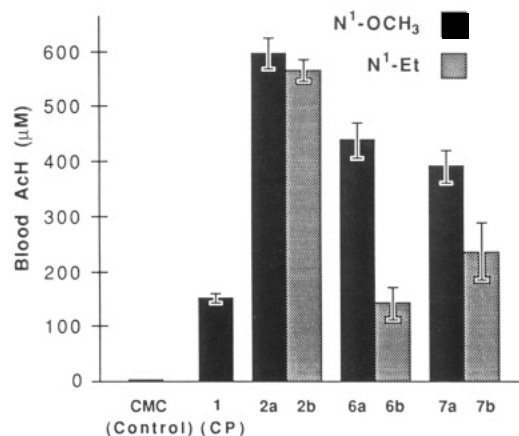


Figure 2. Effect of *N*¹-substituted (arylsulfonyl)ureas on ethanol-derived blood AcH in rats. See the legend to Figure 1 for further details.

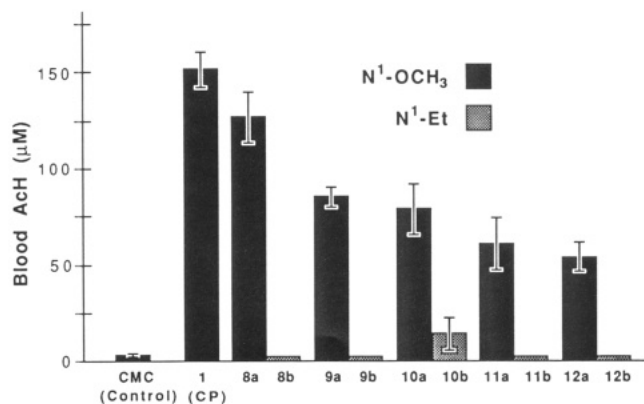


Figure 3. Effect of *N*¹-substituted *N*¹-(arylsulfonyl)carbamates on ethanol-derived blood AcH in rats. The synthesis of 10b is described in ref 6a. See the legend to Figure 1 for further details.

(Chart Ib; Figure 3). It can be seen that regardless of the size of the alkyl group on the alkoxy carbonyl moiety, OMe substitution on the sulfonamide nitrogen gave rise to active compounds, whereas the corresponding compounds with the Et group on the sulfonamide nitrogen were uniformly devoid of any in vivo activity.

Our initial goal was to evaluate the AIDH inhibitory activity of compounds such as 5 bearing a free hydroxyl group on the sulfonamide nitrogen as well as their structural analogs, but because of synthetic difficulties, we compromised with the methoxy group at this position, reasoning that metabolic *O*-demethylation would transform 2a to 5 in vivo. That the liver can in fact catalyze this *O*-demethylation reaction is shown in Figure 4. Liver microsomes from phenobarbital pretreated rats were incubated in the presence of cofactors with 2a, as well as with the series of sulfonylcarbamates 9a–12a (compound 8a was omitted because of potential ambiguities), and the formaldehyde liberated after 30 min was measured. While sluggish compared to aminopyrine, the positive control where *N*-demethylation is involved, the data show that *O*-demethylation of such compounds shown in Chart I can be effected in vitro and therefore would be possible in vivo. However, when the *N*¹-OCH₃ compounds 10a, 11a, and 12a were administered to phenobarbital pretreated rats, followed by ethanol challenge 2 h later, blood AcH levels decreased rather than increased (Figure 5). Increased AcH would have suggested that metabolic *O*-demethylation was enhanced in the phenobarbital treated animals. This decreased blood AcH observed with

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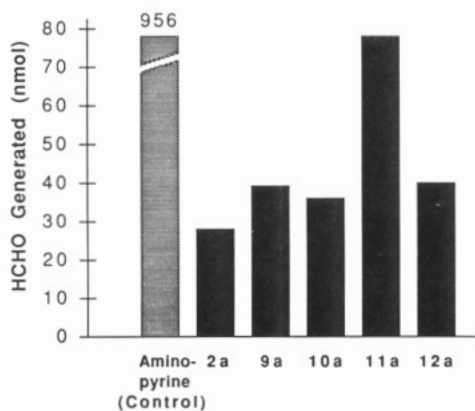


Figure 4. O-Demethylation of N^1 -OMe analogs of CP in vitro by phenobarbital-induced rat liver microsomes measured as formaldehyde generated. The results are given as mean $\pm$ SEM, $n = 3$.

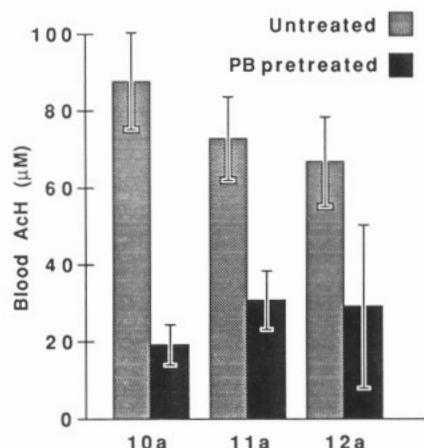


Figure 5. Effect of phenobarbital (PB) pretreatment of rats on ethanol-derived blood AcH by N^1 -methoxy- N^1 -(4-chlorobenzenesulfonyl)carbamates (10a, 11a, and 12a). See the legend to Figure 1 for further details.

phenobarbital pretreatment was not totally unexpected since similar data was seen with CP (1) itself.⁴ Phenobarbital treatment can not only accelerate side chain oxidation, but is also known to induce the cytosolic AIDH of rat liver.¹⁰

Evidence that the bioactivation of 10a, 11a, and 12a in vivo involved initial O-demethylation by the hepatic microsomal enzymes was provided by pretreatment of the rats with 1-benzylimidazole, an inhibitor of cytochrome P-450.¹¹ It can be seen from Figure 6 that 1-benzylimidazole pretreatment completely blocked the bioactivation of 10a, 11a, and 12a, resulting in no inhibition of AIDH in vivo as reflected by no elevation of ethanol-derived blood AcH.

Summarizing the above data, we postulate that compounds such as 15 ($X = \text{NH}; \text{O}$) with a free hydroxyl group on the sulfonamide nitrogen are unstable and would disproportionate in vivo to give 4-chlorobenzenesulfinic acid (16) and an unstable nitrosocarbonyl compound (17) that can readily solvolyze to nitroxyl and, hence, N_2O as the final product (Scheme III). This unique mechanism

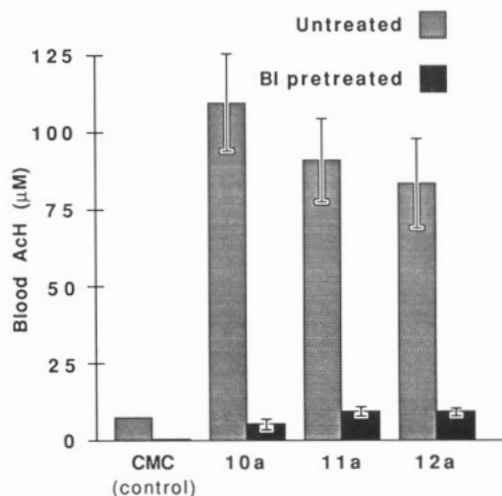


Figure 6. Effect of 1-benzylimidazole (BI) pretreatment of rats on ethanol-derived blood AcH by N^1 -methoxy- N^1 -(4-chlorobenzenesulfonyl)carbamates (10a, 11a, and 12a). See the legend to Figure 1 for further details.

is intriguing in that nitroxyl has also been postulated to be the active metabolite of cyanamide, a clinically used alcohol deterrent agent that inhibits AIDH.¹² This mechanism of disproportionation of 15 is similar to the decomposition mode of Piloty's acid (N -hydroxybenzenesulfonamide) in strong base.¹³ Nitrosocarbonyl compounds exemplified by nitrosobenzoyl (PhCONO) are known to solvolyze readily to give the corresponding carboxylic acid and nitroxyl, with the latter dimerizing to hyponitrous acid ($\text{H}_2\text{N}_2\text{O}_2$) and ultimately decomposing to H_2O and N_2O .¹⁴ We will provide further evidence for Scheme III and nitroxyl formation from similar compounds in a companion paper.

Experimental Section

Organic chemicals were purchased mostly from Aldrich Chemical Company, Milwaukee, WI, while enzymes and reagents for biological assays were purchased from Sigma Chemical Company, St. Louis, MO. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN. Infrared spectra were determined on a Beckman IR-10 or a Digilab FTS-40 infrared spectrophotometer. Some ^1H NMR spectra were obtained on a Varian T-60A (60 MHz) spectrometer, while other ^1H NMR and all ^{13}C NMR spectra were recorded on a Bruker AC-200 (200 MHz), AC-300 (300 MHz), or Gemini 300 (300 MHz) NMR spectrometers. 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. The reaction mixtures and chromatography effluents were monitored by TLC on silica gel GF (Analtech Inc., Newark, DE) using the solvent systems indicated.

General Procedure for the Preparation of [(4-Chlorophenyl)sulfonyl]ethyl(or methoxy)carbamate Alkyl

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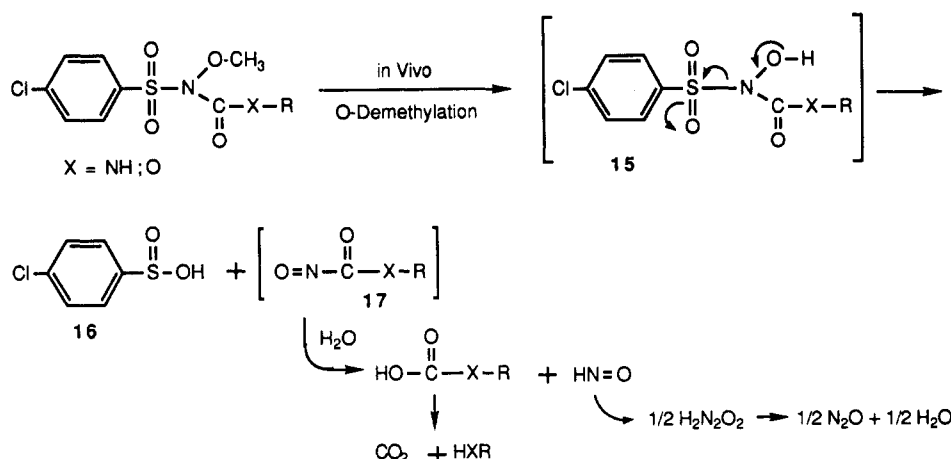
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Scheme III



Esters. [(4-Chlorophenyl)sulfonyl]ethylcarbamic Acid Methyl Ester (8b). NaH (50% in oil, 0.96 g, 0.020 mol) was added to 13c¹⁵ (2.20 g, 0.010 mol) in dimethylacetamide (20 mL) and the mixture stirred for 30 min. Methyl chloroformate (1.89 g, 0.020 mol) was added, and the mixture was stirred for another 30 min. The suspension was then diluted with H₂O (50 mL), and the mixture was extracted with Et₂O (5 × 20 mL). Evaporation of the Et₂O extract gave a residue that was purified by flash chromatography on a silica gel column using hexane-THF (10:1) as eluant. The fractions containing 8b were combined, and the solvent was evaporated. The residue was dissolved in Et₂O, the solution filtered, and the solvent evaporated. The product was recrystallized from Et₂O-hexane to give 8b: colorless crystals; 1.49 g (31% yield); mp 53.1–53.3 °C; IR (KBr, cm⁻¹) 1738 (C=O), 1356 and 1157 (SO₂), 1089 (1,4-disubstituted benzene); ¹H NMR (CDCl₃, 60 MHz, δ) 7.72 (A₂B₂, q, 4 H, J = 9.0 Hz, Δν = 25 Hz, ArH), 3.93 (q, 2 H, J = 6.9 Hz, NCH₂CH₃), 3.72 (s, 3 H, OCH₃), 1.32 (t, ~3 H, J = 6.9 Hz, NCH₂CH₃). Anal. (C₁₀H₁₂ClNO₄S) C, H, N.

[(4-Chlorophenyl)sulfonyl]methoxycarbamic Acid Methyl Ester (8a). This compound was prepared from 13b¹⁶ and methyl chloroformate according to the general procedure above. The flash chromatography eluant was EtOAc-light petroleum (1:5). The product was recrystallized from EtOAc-light petroleum to give 8a: colorless crystals (85% yield); mp 64–65.5 °C; IR (KBr, cm⁻¹) 1749 (C=O), 1383 and 1186 (SO₂), 1092 (1,4-disubstituted benzene); ¹H NMR (60 MHz, δ) 3.8 (s, 3 H, OCH₃), 4.0 (s, 3 H, OCH₃), 7.4–8.0 (q, 4 H, 1,4-disubstituted benzene). Anal. (C₉H₁₀ClNO₅S) C, H, N.

[(4-Chlorophenyl)sulfonyl]methoxycarbamic Acid Ethyl Ester (9a). This compound was prepared from 13b and ethyl chloroformate according to the general procedure above. The flash chromatography eluant was EtOAc-light petroleum (1:5). Fractions (R_f 0.64 in EtOAc-hexane, 1:4) were collected to give a white solid (78% yield); mp 68–69.5 °C; IR (KBr, cm⁻¹) 1745 (C=O), 1382 and 1186 (SO₂); ¹H NMR (60 MHz, δ) 1.27 (t, 3 H, CH₂CH₃), 4.02 (s, 3 H, OCH₃), 4.22 (q, 2 H, OCH₂CH₃), 7.40–7.98 (q, 4 H, 1,4-disubstituted benzene). Anal. (C₁₀H₁₂ClNO₅S) C, H, N.

[(4-Chlorophenyl)sulfonyl]ethylcarbamic Acid Ethyl Ester (9b). This compound was prepared from 13c and ethyl chloroformate according to the general procedure above. The yield of 9b (oil) was 83%: IR (neat, cm⁻¹) 1734 (C=O), 1357 and 1163 (SO₂), 1090 (1,4-disubstituted benzene); ¹H NMR (300 MHz, δ) 7.70 (A₂B₂, q, 4 H, J = 8.7 Hz, Δν = 121 Hz, ArH), 4.15 (q, 2 H, J = 7.1 Hz, OCH₂CH₃), 3.92 (q, 2 H, J = 7.0 Hz, NCH₂CH₃),

1.35 (t, 3 H, J = 7.0 Hz, NCH₂CH₃), 1.21 (t, 3 H, J = 7.1 Hz, OCH₂CH₃). Anal. (C₁₁H₁₄ClNO₄S) C, H, N.

[(4-Chlorophenyl)sulfonyl]methoxycarbamic Acid Propyl Ester (10a). This compound was prepared from 13b and *n*-propyl chloroformate according to the general procedure above. The flash chromatography eluant was EtOAc-hexane (1:4). The yield of 10a (oil) was 78.6%: ¹H NMR (60 MHz, δ) 0.93 (t, 3 H, CH₂CH₃), 1.60 (m, 2 H, CH₂CH₂CH₃), 4.05 (s, 3 H, OCH₃), 4.17 (t, 2 H, OCH₂CH₂CH₃), 7.45–8.05 (q, 4 H, 1,4-disubstituted benzene). Anal. (C₁₁H₁₄ClNO₅S) C, H, N.

[(4-Chlorophenyl)sulfonyl]methoxycarbamic Acid 2-methylpropyl Ester (11a). This compound was prepared from 13b and isobutyl chloroformate according to the general procedure above. The flash chromatography eluant was EtOAc-light petroleum (1:5). The yield of 11a (oil) was 68%: ¹H NMR (200 MHz, δ) 0.89 (d, 6 H, CH(CH₃)₂), 1.93 (m, 1 H, CH₂CH(CH₃)₂), 3.93 (d, 2 H, OCH₂), 4.01 (s, 3 H, OCH₃), 7.47–7.94 (q, 4 H, 1,4-disubstituted benzene); ¹³C NMR (CDCl₃) 18.80 (2 C, CH(CH₃)₂), 27.77 (CH(CH₃)₂), 67.07 (OCH₂CH(CH₃)₂), 74.10 (OCH₃), 129.4 and 130.1 (4 C, aromatic), 135.1 and 141.2 (2 C, substituted aromatic), 152.5 (carbamate C=O). Anal. (C₁₂H₁₆ClNO₅S) C, H, N.

[(4-Chlorophenyl)sulfonyl]ethylcarbamic Acid 2-Methylpropyl Ester (11b). This compound was prepared from 13c and isobutyl chloroformate according to the general procedure above, except that the crude product was distilled at 181–183 °C (2.2 mm). The yield of 11b (oil) was 61%: IR (neat, cm⁻¹) 1732 (C=O), 1360 and 1152 (SO₂), 1091 (1,4-disubstituted benzene); ¹H NMR (300 MHz, δ) 7.70 (A₂B₂, q, 4 H, J = 8.7 Hz, Δν = 121 Hz, ArH), 3.93 (q, 2 H, J = 7.0 Hz, NCH₂CH₃), 3.88 (d, 2 H, J = 6.7 Hz, OCH₂CH), 1.88 (m, 1 H, CH₂CH(CH₃)₂), 1.36 (t, 3 H, J = 7.0 Hz, NCH₂CH₃), 0.87 (d, 3 H, J = 6.7 Hz, CH(CH₃)₂). Anal. (C₁₃H₁₈ClNO₄S) C, H, N.

[(4-Chlorophenyl)sulfonyl]methoxycarbamic Acid 1,1-Dimethylethyl Ester (12a). This compound was prepared from 13c and di-*tert*-butylcarbonyl anhydride according to the general procedure above. The flash chromatography eluant was THF-hexane (1:8). A clear oil was obtained (R_f = 0.78 in THF-hexane, 1:5), which crystallized when stored at -15 °C to give 1.05 g (65.2% yield) of 12a: mp 63–65 °C; IR (KBr, cm⁻¹) 1744 (C=O), 1374, 1153 (SO₂), 1092 (1,4-disubstituted benzene); ¹H NMR (60 MHz, δ) 1.47 (s, 9 H, *tert*-butyl CH₃), 4.03 (s, 3 H, OCH₃), 7.45–8.03 (q, 4 H, aromatic H's, 1,4-disubstituted benzene). Anal. (C₁₂H₁₆ClNO₅S) C, H, N.

[(4-Chlorophenyl)sulfonyl]ethylcarbamic Acid 1,1-Dimethylethyl Ester (12b). This compound was prepared from 13c and di-*tert*-butylcarbonyl anhydride according to the general procedure above. The yield of 12b (low melting solid) was 90%: IR (KBr, cm⁻¹) 1729 (C=O), 1353 and 1156 (SO₂), 1092 (1,4-disubstituted benzene); ¹H NMR (300 MHz, δ) 7.67 (A₂B₂, q, 4 H, J = 8.6 Hz, Δν = 109 Hz, ArH), 3.89 (q, 2 H, J = 7.0 Hz, NCH₂CH₃), 1.37 (s, ~9 H, C(CH₃)₃), 1.35 (t, ~3 H, NCH₂CH₃). Anal. (C₁₃H₁₈ClNO₄S) C, H, N.

General Procedure for the Preparation of 4-Chloro-*N*-ethyl-(or *N*-methoxy)-*N*[(alkylamino)carbonyl]benzene-

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sulfonamides. 4-Chloro-N-ethyl-N-[(methylamino)carbonyl]benzenesulfonamide (7b). A mixture of 13c (1.10 g, 5.0 mmol), triethylamine (1.1 mL, 7.5 mmol), and methyl isocyanate (2.0 mL, 3.4 mmol) placed in each of two capped pressure tubes (5 mL) were heated for 20 h at 75 °C. The cooled solutions were stirred into 1 N HCl (110 mL) to hydrolyze the excess reagent, and after 1 h the mixture was extracted with EtOAc (4 × 20 mL). The extract was dried (Na₂SO₄), and the solvent was evaporated to give an oil. The oil was purified by flash chromatography on a silica gel column using hexane-THF (10:1) as eluant. The fractions were analyzed by TLC using hexane-THF (5:1) as solvent. Fractions containing 7b were combined, and the solvent was evaporated to give an oil. The oil was repurified by prep TLC on 1-mm silica plates eluting with THF-hexane (1:5). The band containing 7b was extracted with THF and evaporated to give 7b: 0.70 g (25% yield); IR (KBr, cm⁻¹) 3411 (NH), 1697 (C=O), 1528 (amide II), 1350 and 1162 (SO₂), 1092 (1,4-disubstituted benzene); ¹H NMR (60 MHz, δ) 7.64 (A₂B₂, q, 4 H, J = 9.0 Hz, Δν = 14 Hz, ArH), 7.12 (b s, 1 H, NH), 3.68 (q, 2 H, J = 6.9 Hz, NCH₂CH₃), 2.90 and 2.82 (2 s, 3 H, NHCH₃), 1.18 (t, 3 H, J = 6.9 Hz, NCH₂CH₃). Anal. (C₁₀H₁₃ClN₂O₃S) C, H, N.

4-Chloro-N-methoxy-N-[(methylamino)carbonyl]benzenesulfonamide (7a). This compound was prepared from 13b and methyl isocyanate according to the general procedure above. The product was not purified by flash chromatography, but was recrystallized from THF-hexane in 30% yield: mp 172–174 °C; ¹H NMR (60 MHz, δ) 2.85 (d, NHCH₃), 4.05 (s, OCH₃), 6.2 (broad s, NH), 7.45–8.0 (q, 4 H, 1,4-disubstituted benzene). Anal. (C₉H₁₁ClN₂O₄S) C, H, N.

4-Chloro-N-methoxy-N-[(propylamino)carbonyl]benzenesulfonamide (2a). This compound was prepared from 13b and n-propyl isocyanate according to the general procedure above. The flash chromatography eluant was EtOAc-hexane (1:4). The product was recrystallized from EtOAc-light petroleum to yield white needles: 1.4 g (63.3% yield); mp 89–90 °C; IR (KBr, cm⁻¹) 3325 (N—H), 1708 (urea C=O), 1377 and 1180 (SO₂); ¹H NMR (90 MHz, δ) 0.82 (t, 3 H, CH₃CH₂), 1.45 (m, 2 H, CH₂CH₂CH₃), 3.14 (q, 2 H, CH₂CH₂NH), 3.98 (s, 3 H, OCH₃), 6.23 (broad s, 1 H, NH), 7.43–7.93 (q, 4 H, ArH). Anal. (C₁₁H₁₅ClN₂O₄S) C, H, N.

Methylpropylcarbamoyl Chloride. A solution of methylpropylamine (10.0 g, 0.137 mol) and pyridine (10.8 g, 0.137 mol) in CH₂Cl₂ (200 mL) was placed in a flask cooled in an ice-salt bath and equipped with a dry ice condenser. A solution of triphosgene (13.5 g, 0.046 mol) was added with stirring at such a rate that the temperature was maintained at less than 4 °C. After 30 min, the ice bath was removed and the solution was allowed to stand overnight. The orange solution was evaporated to near dryness and the semisolid residue was diluted with Et₂O (100 mL) and the mixture was filtered. The filtrate was decolorized with charcoal and evaporated to give an amber liquid which was vacuum distilled. The fraction with bp 96.0–98.0 °C (26 mm) was collected to give a colorless liquid, 8.29 g (45% yield). IR (CCl₄, cm⁻¹): 1740 (C=O); ¹H NMR (60 MHz, δ) 0.93 (t, 3 H, CH₃CH₂), 1.65 (m, 2 H, CH₂CH₂), 3.05 and 3.12 (2 s, 3 H, CH₃N), 3.40 and 3.42 (2 t, 2 H, CH₂, N); n_D²⁰ 1.4517. Anal. (C₆H₁₀ClNO) C: calcd, 44.29; found, 45.00; H, N. This material was used directly without further purification.

4-Chloro-N-ethyl-N-[(methylpropylamino)carbonyl]benzenesulfonamide (6b). NaH (50% in oil, 1.08 g, 0.023 mol) was added to 13c (3.30 g, 0.015 mol) in dimethylacetamide (30 mL) and the mixture stirred for 120 min. Methylpropylcarbamoyl chloride (3.06 g, 0.023 mol) was added, and the mixture was stirred overnight. The suspension was then diluted with H₂O (70 mL), and the mixture was extracted with Et₂O (4 × 20 mL). Evaporation of the Et₂O extract gave a residue that was purified by flash chromatography on a silica gel column using hexane-THF (10:1) as eluant. Fractions containing 6b were combined, and the solvent was evaporated. The residue separated into two phases. TLC showed the presence of two impurities. The oil was purified by flash chromatography using hexane-CH₂Cl₂ (4:1) as eluant. Fractions containing pure 6b were combined, and the solvent was evaporated. The residue was dissolved in Et₂O, the solution filtered, and the solvent evaporated to constant weight to give a colorless oil: 1.49 g (31% yield); IR (neat, cm⁻¹)

1685 (C=O), 1353 and 1177 (SO₂), 1089 (1,4-disubstituted benzene); ¹H NMR (300 MHz, δ) 7.64 (A₂B₂, q, 4 H, J = 8.7 Hz, Δν = 93, ArH), 3.46 (t, 2 H, J = 7.5 Hz, NCH₂CH₂), 3.18 (q, 2 H, J = 7.2 Hz, NCH₂CH₃), 3.17 (s, 3 H, NCH₃), 1.67 (m, 2 H, NCH₂CH₂CH₃), 1.09 (t, 3 H, J = 7.2 Hz, NCH₂CH₃), 0.96 (t, 3 H, J = 7.4 Hz, NCH₂CH₂CH₃). Anal. (C₁₃H₁₉ClN₂O₃S) C, H, N.

4-Chloro-N-methoxy-N-[(methylpropylamino)carbonyl]benzenesulfonamide (6a). This compound was prepared from 13b and methylpropylcarbamoyl chloride according to the procedure for 6b above. The flash chromatography solvent was THF-hexane (1:10). The fractions containing product were further purified by prep TLC on 1-mm silica plates eluted with THF-hexane (1:5). CH, and N analysis indicated that this product retained hexane. The oil was therefore repurified by prep TLC on 1-mm silica plates eluting with CH₂Cl₂. The yield of 6a (oil) was 39%: IR (neat, cm⁻¹) 1709 (C=O), 1365 and 1173 (SO₂), 1089 (1,4-disubstituted benzene); ¹H NMR (60 MHz, δ) 0.97 (t, 3 H, CH₂CH₂CH₃), 1.61 (m, 2 H, CH₂CH₂CH₃), 3.20 (s, 3 H, NCH₃), 3.50 (t, 2 H, NCH₂), 3.93 (s, 3 H, OCH₃), 7.45–7.91 (q, 4 H, 1,4-disubstituted benzene). Anal. (C₁₂H₁₇ClN₂O₄S) 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 Animal Study Committee of the Minneapolis VA Medical Center.

Drug Administration Protocol. The compounds were suspended in 2% aqueous (carboxymethyl)cellulose (CMC) and administered at a dose of 1.0 mmol/kg, ip, at zero time to fasted (24 h) male rats of Sprague-Dawley descent (BioLab, Inc., St. Paul, MN), weighing 185–225 g. Ethanol (2.0 g/kg, ip) was given at 2 h, and the animals were sacrificed 1 h subsequent to each ethanol dose for measurement of blood AcH. CP-treated animals served as positive controls. Phenobarbital as its sodium salt was administered as a 0.1% solution in the drinking water for 6 days, followed by water 24 h prior to the experiment. 1-Benzylimidazole (75 mg/kg, ip) was administered 2 h prior to test drug administration.

Blood Acetaldehyde Determination. Blood AcH levels in treated and control animals were measured 1 h after the administration of ethanol in treated and control animals as previously described.¹⁷

Determination of Formaldehyde Produced by Microsome-Catalyzed O-Demethylation.¹⁸ The test compounds were dissolved in acetone (192 μmol/mL), and an aliquot (final concentration 2 mM) was added to a 50-mL Ehrlemeyer flask and preincubated at 37 °C for 30 min, after which time 10 mL of the incubation mix [containing 80 mM potassium phosphate buffer (pH 7.4), 2 mM NADP, 8 mM nicotinamide, 2.5 mM glucose-6-phosphate, 4 mM semicarbazide (pH 7.0), 16.5 mM KCl, 4 mM MgCl₂, and H₂O] was added and the reaction was initiated by addition of 1.0 mL of glucose-6-phosphate dehydrogenase (40 units/mL) and 0.5 mL of rat liver microsomes. The reaction mixture was incubated at 37 °C for 30 min, and a 6-mL aliquot was taken and added to 2 mL of 15% ZnSO₄, followed by addition of 2 mL of saturated Ba(OH)₂ solution. The protein precipitate was sedimented by centrifugation, a 5-mL aliquot of the supernatant was removed, and 2 mL of Nash reagent (30 g NH₄OAc, 0.4 mL of acetylacetone brought up to 100 mL with H₂O) was added. The mixture was incubated at 55 °C for 30 min, and the absorbance at 412 nm was measured for CH₂O determination.

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Acknowledgment. This work was supported in part by the Department of Veterans Affairs and in part by ADAMHA Grant 1 R01-AA07317. We thank Dr. E. G. DeMaster for his expert help in sacrificing the animals for blood collection.

Registry No. 1, 94-20-2; 2a, 143065-09-2; 2b, 117048-15-4; 3, 98922-58-8; 4, 120789-42-6; 5, 143065-10-5; 6a, 143065-11-6; 6b, 143065-12-7; 7a, 143065-13-8; 7b, 143065-14-9; 8a, 143065-15-0; 8b, 143065-16-1; 9a, 143065-17-2; 9b, 143065-18-3; 10a, 143065-

19-4; 10b, 120789-47-1; 11a, 143065-20-7; 11b, 143065-21-8; 12a, 143065-22-9; 12b, 143065-23-0; 13a, 50695-53-9; 13b, 143076-07-7; 13c, 6318-34-9; 14, 143076-08-8; 16, 100-03-8; methyl chloroformate, 79-22-1; ethyl chloroformate, 541-41-3; *n*-propyl chloroformate, 109-61-5; isobutyl chloroformate, 543-27-1; di-*tert*-butylcarbonic anhydride, 34619-03-9; methyl isocyanate, 624-83-9; *n*-propyl isocyanate, 110-78-1; methylpropylamine, 627-35-0; methylpropylcarbamoyl chloride, 51493-02-8; aldehyde dehydrogenase, 9028-86-8.