# $N^1$-Alkyl-Substituted Derivatives of Chlorpropamide as Inhibitors of Aldehyde Dehydrogenase

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On the basis of an earlier observation that the $N^1$-ethyl derivative of the hypoglycemic agent chlorpropamide (CP) inhibited aldehyde dehydrogenase (AlDH) in rats without producing hypoglycemia, we undertook a structure–activity study to assess the effect of altering the alkyl substituents at $N^1$ and $N^3$, as well as substituting O for N at the latter position, and evaluated these analogues for their effect on AlDH in vivo and in vitro. Our results suggest that only those CP analogues that can release alkyl isocyanates nonenzymatically inhibited AlDH. Increasing the steric bulk of the $N^1$-alkyl substituent enhanced isocyanate formation and AlDH inhibition. CP analogues that lacked the NH group at $N^3$ or were otherwise incapable of alkyl isocyanate release were inactive.

Ethanol–drug interactions manifested by elevated blood acetaldehyde (AcH) levels are consequences of the inhibition of aldehyde dehydrogenase (AlDH, EC 1.2.1.3), the enzyme that catalyzes the conversion of the first metabolic degradation product of ethanol, viz., acetaldehyde, to acetate.[1] Such drug–alcohol reactions are reminiscent of the disulfiram–ethanol reaction (DER),[2] a syndrome characterized by a multitude of adverse physiological reactions upon ethanol ingestion, thereby leading to alcohol avoidance. Studies on the mechanism of such ethanol–drug reactions may therefore provide insights into the molecular basis for AlDH inhibition as well as the structural requirements for such inhibition. This can further lead to the design of second generation AlDH inhibitors that might potentially be useful as alcohol deterrent agents for the clinical treatment of alcoholism.

The oral hypoglycemic agent chlorpropamide (CP, **1a**) and, to a lesser extent, its structural analogue tolbutamide have been known to elicit a chlorpropamide–alcohol flushing (CPAF) reaction in humans characterized by facial flushing and elevated blood AcH in flushers relative to nonflushers.[3] The biochemical–pharmacological basis for this CP–alcohol reaction in rodents (rats, mice) is reflected by the inhibition of the low-$K_m$ hepatic mitochondrial AlDH (class II AlDH) and, as a consequence, a quantum increase in ethanol-derived blood AcH levels.[4] Although the precise molecular mechanism of inhibition of AlDH by CP is not known, metabolic activation must be involved in vivo, since CP is inactive as an AlDH inhibitor in vitro.[4] In our studies designed to elucidate the possible bioactivation mechanisms of CP, we concluded that oxidative or conjugative metabolism of the $N^1$-sulfonamide nitrogen must play a role, since the oxidation of the *n*-propyl side chain appeared to represent detoxication rather than bioactivation reactions.[4a] Accordingly, in order to block direct metabolic functionalization or conjugation at this position, an ethyl group was substituted for H on this $N^1$-H. In contrast to expectations, $N^1$-ethylchlorpropamide ($N^1$-EtCP, **1c**) proved to be several times more active than CP in elevating ethanol-derived blood AcH in rats.[5] Moreover, **1c** was devoid of hypoglycemic effect when tested in rats,[5] making it a good candidate for potential use in alcohol deterrent therapy.

Encouraged by these results, we undertook a structure–activity study in order to gain insights on the mechanism of AlDH inhibition by **1c**, vis-a-vis CP itself. Our approach was to alter the alkyl substitutent at the $N^1$- as well as the $N^3$-position, and also to substitute O for N at the latter position. Our results suggest that as the steric bulk of the $N^1$ substitutent is increased, there is a greater tendency for the resulting molecule to eliminate *n*-propyl isocyanate—a potent inhibitor of AlDH—by a mechanism that requires the presence of a free N-H group at $N^3$. The implications of these findings will be applied to a discussion of the possible bioactivation mechanism for CP.

## Chemistry

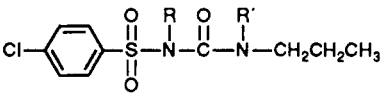
The various $N^1$-alkyl-substituted derivatives of **1a**, viz., **1b–i** (see Chart I), were prepared by the reaction of *n*-propyl isocyanate with the corresponding *N*-alkyl-4-chlorobenzenesulfonamides. The latter were in turn prepared (when not commercially available) by simple acylation of the corresponding amines with 4-chlorobenzenesulfonyl chloride. Noteworthy was the observation that as the steric bulk at the $N^1$-position of the product **1** increased, the reaction with *n*-propyl isocyanate became more sluggish and the yields of the desired products diminished. Indeed, for **1h** (R = *tert*-butyl), the yield was a meager 13% due to steric hindrance and the propensity of the reaction to reverse itself. The action of 4-chlorobenzenesulfonyl isocyanate on methylpropylamine gave **2**.[6] Compounds **3a–c** and **4** were prepared in similar manner as for **1** by the reaction of *tert*-butyl isocyanate or methyl isocyanate (for **4**) on the corresponding *N*-alkyl-4-chlorobenzenesulfonamide. The sulfonylcarbamate **5** was prepared by acylation of *N*-ethyl-4-chlorobenzenesulfonamide with *n*-propyl chloroformate.

## Biological Results

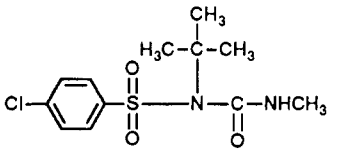
The elevation of blood AcH levels in rats pretreated with the $N^1$-alkyl-substituted CP derivatives **1b–i**, followed by an ethanol challenge 2 h later, is shown in Figure 1. It can be seen that, with the exception of **1i**, $N^1$-alkyl substitution on CP produced derivatives that were more active than CP itself, with **1c** (R = Et), **1d** (R = *n*-propyl), **1e** (R = isopropyl), and **1h** (R = *tert*-butyl) giving rise to 2–3-fold higher levels of blood AcH when measured 3 h post drug administration. Compound **1h** was still active at 16 h.

(1) Anonymous *Med. Lett.* **1975**, *17*, 17.
(2) Kitson, T. M. *J. Stud. Alcohol* **1977**, *38*, 96.
(3) (a) Editorial, *Br. Med. J.* **1964**, *2*, 586. (b) Ohlin, H.; Jerntorp, P.; Bergstrom, B.; Almer, L.-O. *Br. Med. J.* **1982**, *285*, 838. (c) Jerntorp, P.; Ohlin, H.; Bergstrom, B.; Almer, L.-O. *Diabetes* **1981**, *30*, 788. (d) Barnett, A. H.; Gonzales-Auvert, C.; Pyke, D.; Saunders, J. B.; Williams, R.; Dickenson, C.; Rawlins, M. D. *Br. Med. J.* **1981**, *283*, 939. (e) Johnston, C.; Saunders, J. B.; Barnett, A. H.; Ricciardi, B. R.; Hopkinson, D. A.; Pyke, D. A. *Clin. Sci.* **1986**, *71*, 513.
(4) (a) Nagasawa, H. T.; DeMaster, E. G.; Kwon, C.-H.; Fraser, P. S.; Shirota, F. N. *Alcohol* **1985**, *2*, 123. (b) Little, R. G.; Peterson, D. R. *Toxicol. Appl. Pharmacol.* **1985**, *80*, 206.
(5) Nagasawa, H. T.; Elberling, J. A.; Shirota, F. N.; DeMaster, E. G. *Alcoholism, Clin. Exp. Res.* **1988**, *12*, 563.
(6) Nagasawa, H. T.; Smith, W. E.; Kwon, C.-H.; Goon, D. J. W. *J. Org. Chem.* **1985**, *29*, 4993.

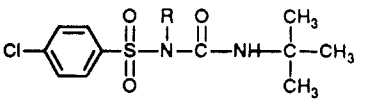
**Chart I**



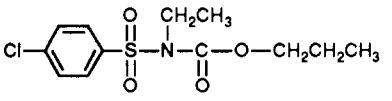
| | R | R' |
|---|---|---|
| **1a** | H | H |
| **1b** | $CH_3$ | H |
| **1c** | $C_2H_5$ | H |
| **1d** | $n$-$C_3H_7$ | H |
| **1e** | $i$-$C_3H_7$ | H |
| **1f** | $n$-$C_4H_9$ | H |
| **1g** | $i$-$C_4H_9$ | H |
| **1h** | $t$-$C_4H_9$ | H |
| **1i** | $CH_2(CH_3)_3$ | H |
| **2** | H | $CH_3$ |



**4**



| | R |
|---|---|
| **3a** | H |
| **3b** | $CH_3$ |
| **3c** | $C_2H_5$ |



**5**

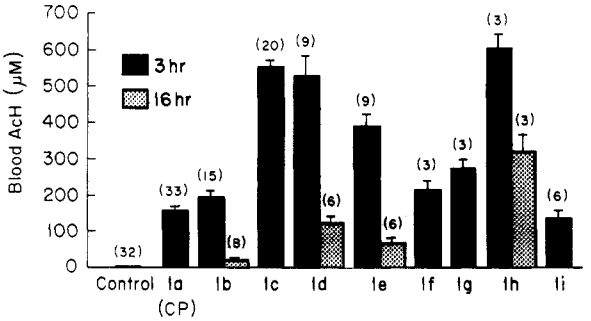



**Figure 1.** Ethanol-derived blood AcH in rats given $N^1$-alkyl-substituted derivatives of CP. These compounds all have a free N-H group on $N^3$. See Experimental Section for details. The N's are indicated within the parentheses. $p < 0.05$ for **1e** at 16 h and $> 0.05$ for **1b** at 16 h when compared to control. All other values are different from control at the 0.01 level.

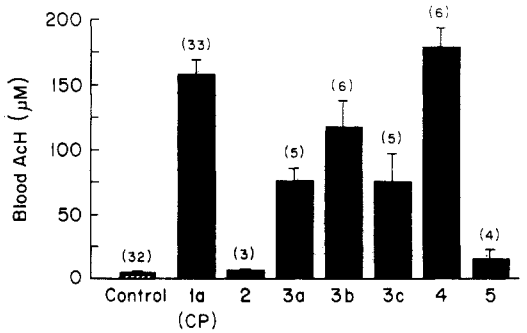



**Figure 2.** Ethanol-derived blood AcH in rats given CP analogues with various substitutions on $N^1$ and $N^3$. See legend to Figure 1 for further details.

The series of CP analogues with the *tert*-butyl group at the $N^3$-position (**3a–c**), as well as 1-*tert*-butyl-3-methyl-[(4-chlorophenyl)sulfonyl]urea (**4**, isomeric with **3b**), raised ethanol-derived blood AcH statistically well above controls (Figure 2), but not to the extent of the CP derivatives evaluated in Figure 1. Of considerable mechanistic significance, the CP analogue that lacked a free $N^3$-H group, viz., **2**, or the sulfonylcarbamate **5**, was completely devoid of activity, and blood AcH levels were indistinguishable from those of control rats given ethanol alone (Figure 2).

We had shown earlier that the elevation of blood AcH in rats elicited by **1c** when followed by an ethanol dose was a consequence of the inhibition of the class II AlDH of liver, and both phenomena were dose-related with respect to **1c**.[5] A more convenient enzyme for the screening of a large variety of potential AlDH inhibitors, and one that has been used successfully as a prognosticator of in vivo activity in the past,[7] is the commercially available yeast AlDH. The inhibition of this enzyme in vitro by 1.0 mM concentrations of most of the CP analogues evaluated in

**Table I.** Inhibition of Yeast AlDH in Vitro by CP Analogues

| CP analogue (1.0 mM) | $N$ | yeast AlDH activity: NADH formation, nmol/min | yeast AlDH activity: % inhibition |
|---|---|---|---|
| none (control) | 5 | 14.8 ± 0.4 | 0 |
| CP (**1a**) | 3 | 14.5 ± 0.3[a] | 2.0 |
| **1b** | 3 | 0.6 ± 0.02 | 99.6 |
| **1c** | 4 | 1.4 ± 0.7 | 98.6 |
| **1d** | 3 | 1.3 ± 0.1 | 91.1 |
| **1e** | 2 | 0.0 | 100 |
| **1f** | 1 | 0.0 | 100 |
| **1g** | 1 | 0.0 | 100 |
| **1h** | 3 | 0.0 | 100 |
| **1i** | 3 | 0.0 | 100 |
| **3c** | 3 | 2.7 ± 0.4 | 97.3 |
| **2** | 3 | 15.0 ± 0.8[a] | 0 |
| **5** | 3 | 14.6 ± 0.4[a] | 1.4 |

[a] Not significantly different from control.

**Table II.** Inhibition of Class II AlDH in Intact and Osmotically Disrupted Rat Liver Mitochondria by **1b**

| | AlDH Activity: nmol of AcH oxidized/(min·mg of protein) | AlDH Activity: % inhibition |
|---|---|---|
| Intact Mitochondria[a] | | |
| control (buffer + acetone) | 26.4 ± 0.75 | 0 |
| CP (**1a**) | 28.3 ± 0.35 | 0 |
| **1b** | 13.4 ± 0.97[b] | 48.9 |
| Osmotically Disrupted Mitochondria[a,c] | | |
| control (buffer + acetone) | 72.0 ± 1.63 | 0 |
| CP (**1a**) | 73.8 ± 1.36 | 0 |
| **1b** | 19.0 ± 2.52[b] | 73.7 |

[a] The drugs (5 μL of a 200 mM solution in acetone) were added to give a final concentration of 1 mM. [b] $p < 0.001$. [c] Sucrose was omitted from the reaction mixture, and $NAD^+$ was added to a final concentration of 0.1 mM.

(7) DeMaster, E. G.; Shirota, F. N.; Nagasawa, H. T. *Biochem. Biophys. Res. Commun.* **1984**, *122*, 358. (b) DeMaster, E. G.; Shirota, F. N.; Nagasawa, H. T. *Alcohol* **1985**, *2*, 117.

**Scheme I**

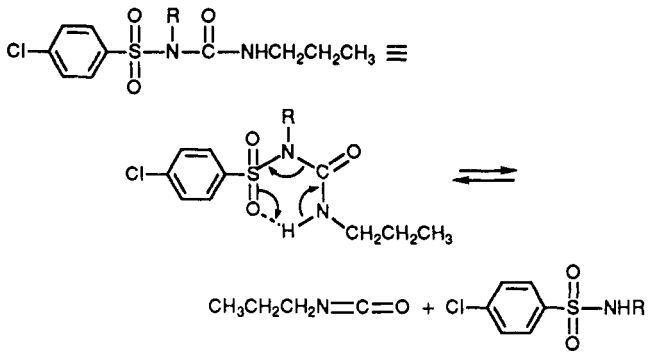


**Table III.** Inhibition of Yeast AlDH in Vitro by Some Alkyl Isocyanates[a]

| | concn, μM[b] | yeast AlDH Activity: NADH formation, nmol/min | yeast AlDH Activity: % inhibition |
|---|---|---|---|
| buffer | | 14.1 | 0 |
| acetone (vehicle) | | 14.0 | 0.5 |
| *n*-propyl isocyanate | 100 | 0.0 | 100.0 |
| *n*-propyl isocyanate | 10 | 0.2 | 98.5 |
| *n*-propyl isocyanate | 1 | 12.9 | 8.8 |
| *n*-butyl isocyanate | 100 | 0.0 | 100.0 |
| *n*-butyl isocyanate | 10 | 0.2 | 98.9 |
| *n*-butyl isocyanate | 1 | 11.2 | 20.8 |
| cyclohexyl isocyanate | 100 | 0.0 | 100.0 |
| cyclohexyl isocyanate | 10 | 0.5 | 96.5 |
| cyclohexyl isocyanate | 1 | 12.4 | 12.1 |

[a] Experimental details were as described for Table I except that the isocyanates were added as acetone solutions, 1.0 mM $NAD^+$ was present in the primary mixture, and the primary reaction was carried out at 0 °C. [b] Final concentration of isocyanate in primary reaction mixture.

vivo (Figures 1 and 2) is documented in Table I. The inhibition of the matrix-bound class II AlDH in intact and osmotically disrupted rat liver mitochondria by the first member of this series, viz., **1b**, is shown in Table II. Several conclusions can be reached by analysis of these data: (a) yeast AlDH is a sensitive alternative to the nonsolubilized rat liver mitochondrial enzyme for the screening of these CP analogues; (b) CP itself is not active in vitro, whereas the $N^1$-alkyl-substituted analogues are highly inhibitory without the necessity for prior bioactivation; (c) as the steric bulk on $N^1$ is increased, 1.0 mM concentrations of the analogues completely and uniformly inhibit the enzyme; and (d) CP analogues without a free $N^3$-H group, such as **2** or the oxygen-bridged analogue **5**, are inactive in this system, in complete accord with the in vivo data of Figure 2.

On the basis of the above results, we postulated that the $N^1$-alkyl derivatives of CP must be slowly and nonenzymatically decomposing in aqueous solutions at physiologic pH to liberate the reactive *n*-propyl isocyanate and the corresponding *N*-alkyl-4-chlorobenzenesulfonamide in a reversal of the condensation reaction (Scheme I).[8] Compounds **2** and **5** are incapable of releasing an alkyl isocyanate via the mechanism depicted and hence would be inactive—as observed. Other alkyl isocyanates, e.g., *tert*-butyl-NCO or MeNCO, could also be released, e.g., from **3** or **4**. In line with this hypothesis, alkyl isocyanates, exemplified by *n*-propyl isocyanate, *n*-butyl isocyanate, and cyclohexyl isocyanate, were found to be highly potent inhibitors of yeast AlDH, with 10 μM concentrations in the incubation media virtually eliminating the activity of this enzyme after 10 min, even at 0 °C (Table III).

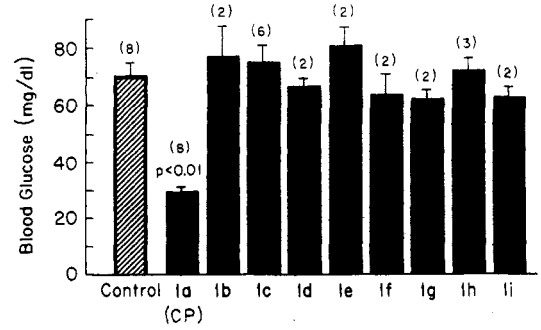



**Figure 3.** Fasting blood glucose levels in rats 4 h after administration of $N^1$-alkyl-substituted CP derivatives. *p* values as indicated; all others are not significantly different from the control.

Since $N^1$-EtCP (**1c**), in contrast to CP, did not elicit hypoglycemia in rats,[5] it was of interest to determine whether this was universally true for the other $N^1$-alkyl-substituted CP derivatives of Figure 1. Accordingly, fasting blood glucose levels were measured in rats 4 h after administration of these compounds. These data are displayed graphically in Figure 3. Except for CP where blood glucose levels fell 43%, the 4-h blood glucose levels of rats treated with the $N^1$-alkyl-substituted CP derivatives were statistically not different from those of control animals given vehicle alone.

## Discussion

The pharmacological interaction between ethanol and the sulfonylurea hypoglycemic agents has not been extensively investigated, and clear understanding of its molecuar mechanism cannot be discerned from the early literature. Podgainy and Bressler[9] claimed that CP was a noncompetitive inhibitor of liver AlDH, but more recent evidence,[4] corroborated by the data presented in Table I, indicates that the sulfonylurea drugs do not inhibit AlDH directly but require metabolic activation in vivo. We have further shown that side-chain-hydroxylated metabolites of CP (or of tolbutamide) or their dehydrogenated or hydrolysis products can be eliminated from consideration as inhibititors of AlDH on the basis of direct evidence with synthesized compounds.[4a] If it is assumed that the (relatively) metabolically stable aromatic (4-chlorophenyl)-sulfonyl group is not involved in the bioactivation process, the only remaining position would be the $N^1$-sulfonamide nitrogen, which, theoretically, can be further functionalized and/or conjugated by reactions that are peculiar to xenobiotic substances.[10]

The present observation that (a) synthetic $N^1$-alkyl-substituted CP derivatives are uniformly good inhibitors of AlDH and do not require bioactivation (Figure 1 and Table I), (b) CP analogues that lack an $N^3$-H group are totally inactive, and (c) *n*-propyl isocyanate is a highly potent inhibitor of yeast AlDH (Table III) led to the postulation of a cyclic transition-state intermediate for compounds such as **1b–i** that would favor the release of *n*-propyl isocyanate (Scheme I). That such a mechanism is feasible comes from our observation that the most sterically hindered member of this group, viz., **1h**, is the most difficult to prepare due to the tendency for the conden-

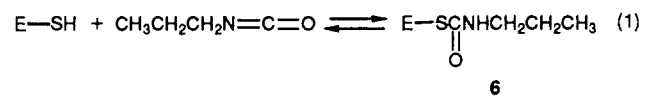
(8) When **1h** was incubated at 37 °C in a pH 7.5 phosphate buffer/DMSO (1:1) solution, *N*-*tert*-butyl-4-chlorobenzenesulfonamide was rapidly produced over time as detected by TLC. Compound **1h** was completely decomposed after 20 min.

(9) Podgainy, H.; Bressler, R. *Diabetes* **1968**, *17*, 679.
(10) Testa, B.; Jenner, P. *Drug Metabolism: Chemical and Biochemical Aspects*; Marcel Dekker: New York, 1976, pp 3–235.

sation (forward) reaction to reverse itself.[8] Moreover, it is known that **1b** is thermally unstable and decomposes to *N*-methyl-4-chlorobenzenesulfonamide (and presumably, *n*-propyl isocyanate) under gas chromatography conditions.[11]

While the evidence is merely circumstantial at this time, this hypothesis explains all of the biological data for the CP analogues synthesized for this structure–activity study. With regard to the actual bioactivation process that must take place for CP in vivo, *enzymatic tert*-butylation or isopropylation reactions are, of course, unknown in biochemistry or pharmacology, and such a mechanism can be summarily dismissed. On the other hand, enzymatic *methylation* on the $N^1$-position to produce **1b**, a derivative of CP that is active as an AlDH inhibitor without requiring metabolism (Figure 1 and Tables II and III), is a possibility that must be seriously entertained. However, this mechanism cannot be considered to be fully established until more direct evidence for the existence of this bioactivation pathway can be demonstrated. It is of interest that acetylation on the $N^1$-sulfonamide nitrogen has also been postulated as a bioactivation mechanism for CP, since such a reaction leads to the spontaneous formation of *n*-propyl isocyanate.[6]

Although the exact position of the active site on the peptide chain of AlDH or the nature of the functional group involved is presently in dispute, it has generally been assumed that one or more cysteine sulfhydryl groups play key roles.[12] Reaction of isocyanates with RSH groups produces thiocarbamates, which are known to be unstable at pH 7 or higher by virtue of reversibility.[13,14] Such a reaction of *n*-propyl isocyanate with the SH group at the active site of AlDH should therefore lead to an E–I product with a thiocarbamate structure **6** (eq 1). The reversibility



(1)

**6**

of this reaction at physiological pH and temperature—as reflected by the reversibility of enzyme inhibition with time, or on dilution—has not yet been studied. Nevertheless, use of the CP analogues described here to probe the active site of AlDH appears to be quite feasible, since *S*-(butylcarbamoyl)cysteinyl peptides and *S*-(butylcarbamoyl)cysteine have been isolated from proteolytic digests of yeast alcohol dehydrogenase after treatment with butyl isocyanate, an active-site-specific inhibitor of this enzyme.[14]

## Experimental Section

Melting points were determined on a Fisher-Johns or Mettler FP2 melting point apparatus and are corrected to reference standards. $^1H$ and $^{13}C$ NMR spectra were recorded on a Varian T-60A and Nicolet NT-300 WB nuclear magnetic resonance spectrometers, respectively, with tetramethylsilane ($Me_4Si$) as internal standard. IR spectra were taken as KBr pellets or in $CH_2Cl_2$ on a Beckman IR-10 infrared spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Chlorpropamide (**1a**) was prepared according to a general procedure.[6] (4-Chlorophenyl)sulfonyl isocyanate was purchased from Morton Thiokol, Inc. The reactions and chromatography effluents were monitored by TLC on silica gel GF (Analtech) using the solvent systems indicated.

**General Procedure for the Preparation of Compounds 1. 1-Ethyl-1-[(4-chlorophenyl)sulfonyl]-3-*n*-propylurea (1c).** A mixture of *N*-ethyl-4-chlorobenzenesulfonamide[15] (1.10 g, 5.0 mmol), triethylamine (1.10 mL, 7.5 mmol), and *n*-propyl isocyanate (2.13 g, 25 mmol) was placed into each of four pressure tubes (5 mL). The tubes were capped and heated for 20 h at 75 °C. The cooled solutions were stirred into 1 N HCl (180 mL) to hydrolyze the excess reagent, and after 45 min the mixture was extracted with EtOAc (4 × 30 mL). The extract was dried ($Na_2SO_4$) and the solvent evaporated to give **1c** as an oil. The oil was purified by flash chromatography[16] on a silica gel column using $CH_2Cl_2$ as eluant. The fractions were analyzed by TLC using toluene–EtOAc–HOAc (8:1:1) as solvent. The fractions containing pure **1c** were combined, and the solvent was evaporated. The residue was dissolved in $Et_2O$, the ethereal solution filtered through a filter aid, and the solvent evaporated to constant weight to give **1c** as a colorless oil, 3.39 g (56% yield): $^1H$ NMR ($CDCl_3$) $\delta$ 7.69 ($A_2B_2$, q, 4 H, $J$ = 8 Hz, $\Delta\nu_{AB}$ = 18.0 Hz, ArH), 7.3 (br s, 1 H, N*H*), 3.72 (q, 2 H, $J$ = 7 Hz, NC$H_2$CH$_3$), 3.27 (q, 2 H, $J$ = 7 Hz, NHC$H_2$CH$_2$), 0.73–1.83 (m, 8 H, CH$_2$C$H_2$C$H_3$, CH$_2$C$H_3$); $^{13}C$ NMR ($CDCl_3$, 300 MHz) $\delta$ 11.32, 15.24, 22.79, 41.71, 42.80, 128.13, 129.64, 137.79, 140.16, 151.95. Anal. ($C_{12}H_{17}N_2O_3SCl$) C, H, N.

**1-*n*-Propyl-1-[(4-chlorophenyl)sulfonyl]-3-*n*-propylurea (1d).** This compound was prepared from *N*-*n*-propyl-4-chlorobenzenesulfonamide[15] and *n*-propyl isocyanate according to the general procedure above, except that the flash chromatography eluant was hexane–THF (10:1) and the TLC solvent was hexane–THF (5:1). The yield of **1d** (oil) was 42%: $^1H$ NMR ($CDCl_3$) $\delta$ 7.68 ($A_2B_2$, q, 4 H, $J$ = 8 Hz, $\Delta\nu_{AB}$ = 23.4 Hz, ArH), 7.22 (br s, 1 H, N*H*CH$_2$), 3.63 (t, 2 H, $J$ = 7 Hz, N–C$H_2$CH$_2$), 3.08 (q, 2 H, $J$ = 6 Hz, NHC$H_2$CH$_2$), 1.63 (m, 4 H, C$H_2$CH$_3$), 0.093 (t, 6 H, CH$_2$C$H_3$). Anal. ($C_{13}H_{19}N_2O_3SCl$) C, H, N.

**1-Isopropyl-1-[(4-chlorophenyl)sulfonyl]-3-*n*-propylurea (1e).** This compound was prepared from *N*-isopropyl-4-chlorobenzenesulfonamide[17] and *n*-propyl isocyanate according to the general procedure. The yield of **1e** (oil) was 64%: $^1H$ NMR ($CDCl_3$) $\delta$ 7.65 ($A_2B_2$, q, 4 H, $J$ = 8 Hz, $\Delta\nu_{AB}$ = 17.0 Hz, ArH), 7.03 (br, 1 H, N*H*CH$_2$), 4.38 [m, 1 H, $J$ = 6 Hz, C*H*(CH$_3$)$_2$], 3.2 (q, 2 H, $J$ = 7 Hz, NHC$H_2$CH$_2$), 1.4–1.8 (m, 2 H, CH$_2$C$H_2$CH$_3$), 1.33 [d, 6 H, $J$ = 6 Hz, CH(C$H_3$)$_2$], 0.92 (t, 3 H, $J$ = 6 Hz, CH$_2$C$H_3$). Anal. ($C_{13}H_{19}N_2O_3SCl$) C, H, N.

**1-*n*-Butyl-1-[(4-chlorophenyl)sulfonyl]-3-*n*-propylurea (1f).** This compound was prepared from *N*-*n*-butyl-4-chlorobenzenesulfonamide[3] and *n*-propyl isocyanate according to the general procedure: yield (oil) 29%; $^1H$ NMR ($CDCl_3$) $\delta$ 7.20 ($A_2B_2$, q, 4 H, $J$ = 8 Hz, $\Delta\nu_{AB}$ = 17.9 Hz, ArH), 4.20 (br, 1 H, N*H*CH$_2$), 3.65 (t, 2 H, $J$ = 7 Hz, NC$H_2$CH$_2$), 3.25 (q, 2 H, $J$ = 6 Hz, NHC$H_2$CH$_2$), 1.4 (m, 6 H, C$H_2$CH$_3$, C$H_2$C$H_2$CH$_3$), 1.07 (m, 6 H, CH$_2$C$H_3$). Anal. ($C_{14}H_{21}N_2O_3SCl$) C, H, N.

***N*-Isobutyl-4-chlorobenzenesulfonamide.** This compound was prepared from isobutylamine and 4-chlorobenzenesulfonyl chloride according to Lombardino.[18] The crude product was recrystallized from benzene–heptane to give colorless plates, mp 103 °C (65% yield): $^1H$ NMR ($CDCl_3$) $\delta$ 7.68 ($A_2B_2$, q, 4 H, $J$ = 8 Hz, $\Delta\nu_{AB}$ = 23.4 Hz, ArH), 5.23 (t, 1 H, $J$ = 7 Hz, N*H*CH$_2$), 2.78 (t, 2 H, $J$ = 7 Hz, NHC$H_2$CH), 1.68 [m, 1 H, C*H*(CH$_3$)$_2$], 0.88 [d, 6 H, $J$ = 7 Hz, CH(C$H_3$)$_2$]. Anal. ($C_{10}H_{14}NO_2SCl$) C, H, N.

**1-Isobutyl-1-[(4-chlorophenyl)sulfonyl]-3-*n*-propylurea (1g).** This compound was prepared from *N*-isobutyl-4-chlorobenzenesulfonamide and *n*-propyl isocyanate according to the general procedure: yield (oil) 52%; $^1H$ NMR ($CDCl_3$) $\delta$ 7.63 ($A_2B_2$, q, 4 H, $J$ = 9 Hz, $\Delta\nu_{AB}$ = 18.4 Hz, ArH), 7.12 (br, 1 H, N*H*CH$_2$), 3.52 (d, 2 H, $J$ = 8 Hz, NC$H_2$), 3.23 (q, 2 H, $J$ = 6 Hz, NHC$H_2$CH$_2$), 1.24 [m, 3 H, C*H*(CH$_3$)$_2$, CH$_2$C$H_2$CH$_3$], 0.95 [d and t, 9 H, CH(C$H_3$)$_3$ (the CH$_2$C$H_3$ triplet is buried under the

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doublet)]. Anal. ($C_{14}H_{21}N_2O_3SCl$) C, H, N.

**1-*tert*-Butyl-1-[(4-chlorophenyl)sulfonyl]-3-*n*-propylurea (1h).** *N*-*tert*-Butyl-4-chlorobenzenesulfonamide[18] (2.91 g, 0.012 mol) and triethylamine (22 g, 0.22 mol) were dissolved in *n*-propyl isocyanate (100 g, 1.18 mol), and the solution was heated for 48 h at 75 °C. The solution was then poured into 1 N HCl (1 L) and stirred for 1 h to hydrolyze the excess isocyanate. After extraction with EtOAc (6 × 100 mL), the combined extract was dried ($Na_2SO_4$) and the solvent evaporated in vacuo to give 4.85 g of oil. The oil was dissolved in hexane (20 mL) which, on cooling, yielded crystals of starting material (1.73 g). This was removed by filtration, and the filtrate was evaporated to yield an oil, 3.05 g. The oil was dissolved in THF and applied to six preparative silica gel plates (2 mm) which had been activated by heating for 1 h at 105 °C, and the plates were developed with hexane–THF (4:1). A single wide band, $R_f$ 0.50, was visible when viewed under a 2578-nm UV lamp. After drying, the plates were developed again with the same solvent when the original band separated into two bands, $R_f$ 0.55 and 0.44. The $R_f$ 0.55 bands were scraped off and extracted with THF. The combined extracts were rechromatographed on three preparative TLC plates. The product was crystallized from $CHCl_3$–heptane to give colorless crystals of **1h**, 0.51 g (13% yield), mp 69–72 °C: $^1$H NMR ($CDCl_3$) δ 7.68 ($A_2B_2$, q, 4 H, $J$ = 9 Hz, $\Delta\nu_{AB}$ = 32.3 Hz, ArH), 6.02 (br, 1 H, N*H*$CH_2$), 3.22 (q, 2 H, $J$ = 7 Hz, NHC*H*$_2$$CH_2$), 0.73–1.83 [m, 14 H, C*H*$_2$$CH_3$, C(C*H*$_3$)$_3$, $CH_2$C*H*$_3$]; $^{13}$C NMR ($CDCl_3$, 300 MHz) δ 11.41, 22.19, 29.76, 43.43, 60.66, 129.02, 129.48, 139.12, 140.46, 153.43. Anal. ($C_{14}H_{21}N_2O_3SCl$) C, H, N.

***N*-Neopentyl-4-chlorobenzenesulfonamide.** To a cooled (ice bath) solution of neopentylamine (9.59 g, 0.11 mol) and triethylamine (20.24 g, 0.200 mol) in $CHCl_3$ (50 mL) was added, with stirring, 4-chlorobenzenesulfonyl chloride (21.1 g, 0.100 mol) in $CHCl_3$ (70 mL). The solution was then heated under reflux for 2 h, and the cooled solution was extracted with 3 N HCl (2 × 100 mL). The $CHCl_3$ layer was separated, dried ($Na_2SO_4$), and evaporated to dryness to give a solid residue which was recrystallized from $Et_2O$ to give colorless crystals, 23.4 g (89% yield), mp 119.4–119.9 °C: $^1$H NMR ($CDCl_3$) δ 7.67 ($A_2B_2$, q, 4 H, $J$ = 8 Hz, $\Delta\nu_{AB}$ = 23.4 Hz, ArH), 5.25 (t, 1 H, $J$ = 7 Hz, N*H*$CH_2$), 2.70 (d, 2 H, $J$ = 7 Hz, NHC*H*$_2$), 0.90 [s, 9 H, C(C*H*$_3$)$_3$]. Anal. ($C_{11}H_{16}NO_2SCl$) C, H, N.

**1-Neopentyl-1-[(4-chlorophenyl)sulfonyl]-3-*n*-propylurea (1i).** This compound was prepared from *N*-neopentyl-4-chlorobenzenesulfonamide and *n*-propyl isocyanate according to the general procedure. The crude product was recrystallized from $Et_2O$–petroleum ether to give **1i**, colorless crystals, mp 73.2–76.6 °C (79% yield): $^1$H NMR ($CDCl_3$) δ 7.63 ($A_2B_2$, q, 4 H, $J$ = 8 Hz, $\Delta\nu_{AB}$ = 17.9 Hz, ArH), 6.92 (br t, 1 H, N*H*$CH_2$), 3.67 (s, 2 H, NC*H*$_2$), 3.17 (q, 2 H, $J$ = 6 Hz, NHC*H*$_2$$CH_2$), 1.45 (m, 2 H, C*H*$_2$$CH_3$), 0.98 [s, 9 H, C(C*H*$_3$)$_3$] 0.83 (m, under 0.98 peak, 3 H, $CH_2$C*H*$_3$). Anal. ($C_{15}H_{23}N_2O_3SCl$) C, H, N.

**3-*tert*-Butyl-1-[(4-chlorophenyl)sulfonyl]urea (3a).** (4-Chlorophenyl)sulfonyl isocyanate (4.25 g, 19.5 mmol) in $CH_2Cl_2$ (15 mL) was slowly added to a solution of *tert*-butylamine (1.50 g, 20.5 mmol) in $CH_2Cl_2$ (10 mL) with ice cooling. After being allowed to stand 18 h, the solvent was evaporated in vacuo and the residue was recrystallized from $Et_2O$ to give 3.01 g of **3a**, mp 157.2–158.5 °C (52% yield): $^1$H NMR ($CDCl_3$) δ 7.7 Hz ($A_2B_2$, q, 5 H, $J$ = 8 Hz, $\Delta\nu_{AB}$ = 23.4 Hz, ArH), 6.45 (br s, 1 H, N*H*), 1.30 [s, 9 H, C(C*H*$_3$)$_3$]. Anal. ($C_{11}H_{15}N_2O_3SCl$) C, H, N.

**3-*tert*-Butyl-1-methyl-1-[(4-chlorophenyl)sulfonyl]urea (3b).** This compound was prepared from *N*-methyl-4-chlorobenzenesulfonamide[15] and *tert*-butyl isocyanate according to the general procedure above except that the flash chromatography eluant was $CH_2Cl_2$–hexane (3:2). The product was recrystallized from $Et_2O$–petroleum ether to give **3b**, colorless crystals, mp 71.3–71.7 °C (52% yield): $^1$H NMR ($CDCl_3$) δ 7.67 ($A_2B_2$, q, 4 H, $J$ = 8 Hz, $\Delta\nu_{AB}$ = 17.9 Hz, ArH), 7.12 (br s, 1 H, N*H*), 3.13 (s, 3 H, NC*H*$_3$), 1.37 [s, 9 H, C(C*H*$_3$)$_3$]. Anal. ($C_{12}H_{17}N_2O_3SCl$) C, H, N.

**3-*tert*-Butyl-1-ethyl-1-[(4-chlorophenyl)sulfonyl]urea (3c).** This compound was prepared from *N*-ethyl-4-chlorobenzenesulfonamide and *tert*-butyl isocyanate according to the general procedure. The product was recrystallized from $Et_2O$–petroleum ether to give **3c**, colorless crystals, mp 84.6–85.9 °C (57.2% yield): $^1$H NMR ($CDCl_3$) δ 7.67 ($A_2B_2$, q, 4 H, $J$ = 8 Hz, $\Delta\nu_{AB}$ = 17.9 Hz, ArH), 7.10 (s, 1 H, N*H*), 3.68 (q, 2 H, $J$ = 7 Hz, NC*H*$_2$$CH_3$), 1.35 [s, 9 H, C(C*H*$_3$)$_3$], 1.22 (t, 3 H, $J$ = 7 Hz, NCH$_2$C*H*$_3$). Anal. ($C_{13}H_{19}N_2O_3SCl$) C, H, N.

**1-*tert*-Butyl-3-methyl-1-[(4-chlorophenyl)sulfonyl]urea (4).** This compound was prepared from *N*-*tert*-butyl-4-chlorobenzenesulfonamide and methyl isocyanate according to the general procedure. The product was recrystallized from THF–hexane to give **4**, colorless crystals, mp 155.4–157.2 °C (57.2% yield): $^1$H NMR ($CDCl_3$) δ 7.72 ($A_2B_2$, q, 4 H, $J$ = 8 Hz, $\Delta\nu_{AB}$ = 32.0 Hz, ArH), 6.08 (br s, 1 H, N*H*), 2.88 (d, 3 H, $J$ = 5 Hz, NHC*H*$_3$), 1.55 [s, 9 H, C(C*H*$_3$)$_3$]; $^{13}$C NMR ($CDCl_3$, 300 MHz) δ 28.05, 29.73, 60.76, 129.06, 129.46, 139.18, 140.40, 154.12. Anal. ($C_{12}H_{17}N_2O_3SCl$) C, H, N.

***n*-Propyl *N*-Ethyl-*N*-[(4-chlorophenyl)sulfonyl]carbamate (5).** NaH (50% in oil, 0.96 g, 0.020 mol) was added to *N*-ethyl-4-chlorobenzenesulfonamide (2.20 g, 0.010 mol) in dimethylacetamide (20 mL) and the mixture stirred for 30 min. *n*-Propyl chloroformate (2.45 g, 0.020 mol) was then added, and stirring was continued for 2 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 a residue that was purified by flash chromatography on a silica gel column using hexane–THF (10:1) as eluant. Fractions containing pure **5** were combined, and the solvent was evaporated. The residue was dissolved in $Et_2O$, the solution filtered, and the solvent evaporated to constant weight to give **5** as a colorless oil, 2.96 g (97% yield): $^1$H NMR ($CDCl_3$) δ 7.72 ($A_2B_2$, q, 4 H, $J$ = 8 Hz, $\Delta\nu_{AB}$ = 27.2 Hz, ArH), 3.7–4.4 (m, 4 H, OC*H*$_2$$CH_2$, NC*H*$_2$$CH_2$), 0.63–1.97 (m, 8 H, $CH_2$C*H*$_2$$CH_3$, $CH_2$C*H*$_3$). Anal. ($C_{12}H_{16}NO_4SCl$) 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 Subcommittee of the Minneapolis VA Medical Center.

**Drug Administration Protocol.** The CP analogues were suspended in 2% aqueous (carboxymethyl)cellulose (CMC) and administered at a dose of 1.0 mmol/kg, ip, at zero time to fasted male rats of Sprague-Dawley descent (BioLab, Inc., St. Paul, MN), weighing 185–225 g.[19] Ethanol (2.0 g/kg, ip) was given either at 2 h or at 15 h (Figures 1 and 2). The animals were sacrificed 1 h following each ethanol dose for measurement of blood acetaldehyde. CP-treated animals served as positive control. The numbers of animals used for each drug protocol are given in the figures.

**Blood Acetaldehyde Determination.** Blood AcH levels were measured 1 h after the administration of ethanol in treated and control animals as previously described.[20]

**Effect on CP (1a) and of $N^1$-MeCP (1b) on Rat Liver Mitochondrial AlDH Activity in Vitro.** Mitochondria were isolated from overnight fasted male rats weighing between 200 and 250 g, as previously described.[4a]

The activity of the low-$K_m$ AlDH isozyme was assayed in intact mitochondria by measuring the rate of acetaldehyde disappearance from a closed system by use of head-space gas chromatography as described earlier.[4a] Briefly, the incubation mixture contained 0.25 M sucrose, 5.0 mM $MgSO_4$, 1.0 mM EDTA, 10 mM KCl, 10 mM sodium phosphate (pH 7.5), intact rat liver mitochondria, and the test compound in a total volume of 1.0 mL. Following a 5-min preincubation period at 37 °C, the enzymatic reaction was initiated by the addition of acetaldehyde (200 nmol). After incubation for 5 min at 37 °C the reaction was quenched with cold $HClO_4$ (final concentration 0.5 N), and the sample was stored on dry ice until analyzed. In some incubations, the mitochondria were disrupted by omitting sucrose from the reaction media. In

(19) These $N^1$-substituted CP analogues also appear to have skeletal muscle relaxant properties, since animals receiving these compounds were generally docile, flaccid, and easy to handle. However, they were otherwise alert and did not appear to be sedated.

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these incubations, the rate of acetaldehyde disappearance represents a combination of the activities of the high- and low-$K_m$ AlDH isozymes. AlDH activity is expressed in units of nanomoles of AcH oxidized per minute per milligram of protein. The protein concentration was determined by the method of Lowry et al.[21]

**Effect of CP Analogues on Yeast AlDH Activity (Table I).** Commercial yeast AlDH was preincubated for 10 min at 37 °C in a primary reaction mix containing 1.0 mM of the analogue (prepared in DMSO and added to mix in 5 μL), 0.074 IU of yeast AlDH, and 100 mM potassium phosphate, pH 7.4, in a total volume of 0.1 mL. At 10 min, a 20-μL aliquot of the primary mix was added to a cuvette containing 0.5 mM $NAD^+$, 1.0 mM EDTA, 30% glycerol, and 80 mM potassium phosphate (pH 8.0) in a final volume of 1.0 mL. This secondary reaction was initiated by the addition of benzaldehyde (0.6 μmol). The yeast AlDH activity was determined spectrophotometrically by following the increase in absorbance at 340 nm with time.

**Blood Glucose Determination (Figure 3).** Fasting blood glucose levels were determined 4 h after administration of the CP analogues essentially as described previously.[5]

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# Metabolism of 3-(*p*-Chlorophenyl)pyrrolidine. Structural Effects in Conversion of a Prototype γ-Aminobutyric Acid Prodrug to Lactam and γ-Aminobutyric Acid Type Metabolites

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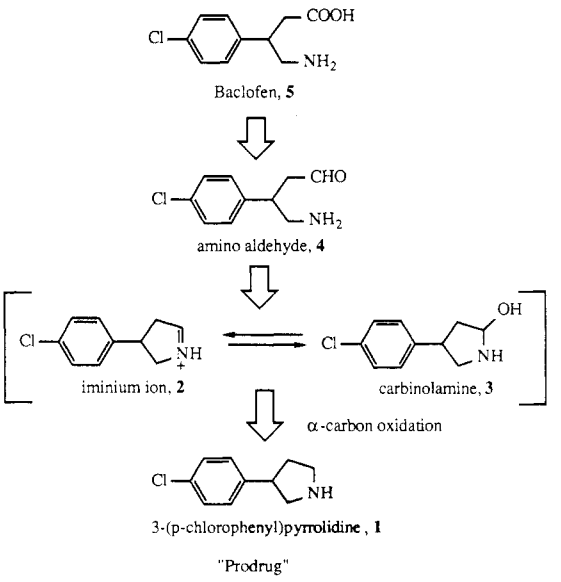
By use of rat liver or brain homogenate supernatants containing microsomes and/or mitochondria, it was found that the prototype GABAergic prodrug [3-(*p*-chlorophenyl)pyrrolidine (**1**)] underwent a series of α-oxidation transformations to a pair of amino acid metabolites and a pair of lactam metabolites [4-amino-3-(*p*-chlorophenyl)butanoic acid, baclofen (**5**); 4-amino-2-(*p*-chlorophenyl)butanoic acid (**10**); 4-(chlorophenyl)pyrrolidin-2-one (**6**); and 3-(*p*-chlorophenyl)pyrrolidine-2-one (**11**)]. With the liver homogenates, the formation of the lactam metabolites was approximately 2 orders of magnitude greater than that of the amino acid metabolites, while with the brain homogenates, the amino acid and lactam pathways were of similar magnitude. For either tissue, for both the lactam and the amino acid series, attack at the less sterically hindered 5-position of the pyrrolidine ring was greater than the attack at the 2-position (**5** > **10** and **6** > **11**) with the exception of the liver homogenate mitochondrial fraction (**6** < **11**). The parenteral administration of the prodrug **1** was found to give detectable brain levels of **5** as well as activity in an isoniazid-induced (GABA-inhibited) convulsion model.

γ-Aminobutyric acid (GABA), first discovered about 40 years ago in the central nervous system (CNS) of a variety of animals,[1] has been shown to be an important CNS neurotransmitter.[2] GABAergic mechanisms have been implicated in analgesia,[3] Alzheimer's disease,[4] cardiovascular function,[5,6] epilepsy,[7,8] Huntington's chorea,[9] Parkinson's disease,[10] and schizophrenia.[11] The greatest amount of research into GABA mechanisms, agonists, and antagonists has been in the area of epilepsy. Studies have shown persons who suffer from a variety of epilepsies to be deficient in brain GABA levels as compared to nonepileptics.[12,13] Also, a number of drugs currently used as anticonvulsants, including barbiturates,[8,14] benzodiazepines,[8,15] hydantions,[8] and valproic acid,[8] have been purported to act through the GABAergic pathway. While GABA itself has not been shown to be useful,[16,17] many

**Scheme I.** Retrometabolic Pathway to Baclofen

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