Novel Prodrugs of Cyanamide That Inhibit Aldehyde Dehydrogenase in Vivo†

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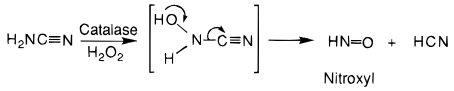
S-Methylisothiourea (**4**), when administered to rats followed by a subsequent dose of ethanol, gave rise to a 119-fold increase in ethanol-derived blood acetaldehyde (AcH) levels—a consequence of the inhibition of hepatic aldehyde dehydrogenase (AlDH)—when compared to control animals not receiving **4**. The corresponding *O*-methylisourea was totally inactive under the same conditions, suggesting that differential metabolism may be a factor in this dramatic difference between the pharmacological effects of *O*-methylisourea and **4** in vivo. The *S*-*n*-butyl- and *S*-isobutylisothioureas (**8** and **9**, respectively) at doses equimolar to that of **4** were nearly twice as effective in raising ethanol-derived blood AcH, while *S*-allylisothiourea (**10**) was slightly less active. However, blood ethanol levels of all experimental groups were indistinguishable from controls. Pretreatment of the animals with 1-benzylimidazole, a known inhibitor of the hepatic mixed function oxidases, followed sequentially by either **8**, **9**, or **10** *plus* ethanol, reduced blood AcH levels by 66−88%, suggesting that the latter compounds were being oxidatively metabolized to a common product that led to the inhibition of AcH metabolism. In support of this, when **8** was incubated in vitro with rat liver microsomes coupled to catalase and yeast AlDH, the requirement for microsomal activation for the inhibition of AlDH activity was clearly indicated. We suggest that S-oxidation is involved and that the *S*-oxides produced in vivo inhibited AlDH directly, or spontaneously released cyanamide, an inhibitor of AlDH. Indeed, incubation of **8** with rat liver microsomes and NADPH gave rise to cyanamide as metabolite, identified as its dansylated derivative. Cyanamide formation was minimal in the absence of coenzyme. Extending the side chain was detrimental, since *S*-benzylisothiourea (**11**) and *S*-*n*-hexadecylisothiourea (**12**) were toxic, the latter producing extensive necrosis of the liver and kidneys when administered to rats.

Introduction

Cyanamide, an alcohol deterrent agent used in Europe, Canada, and Japan, is a potent in vivo inhibitor of the hepatic class 2 (low K_m) aldehyde dehydrogenase (AlDH2)[1] and induces a cyanamide-ethanol reaction (CER)[2] that mimics the disulfiram-ethanol reaction (DER)[3] on consumption of ethanol. This CER is manifested by palpitation, facial flushing, and a general feeling of malaise that ostensibly serves to deter further drinking of alcohol. Cyanamide, unlike disulfiram, has a short duration of action,[4] but like disulfiram, must be bioactivated in vivo since cyanamide itself is inactive in vitro.[5] The enzyme catalase, in the presence of H_2O_2, mediates the oxidation of cyanamide[6] to *N*-hydroxycyanamide,[7] and the latter spontaneously releases nitroxyl,[8] the putative inhibitor of AlDH2 (Scheme 1).

Earlier, we had designed and developed a series of acyl, N-protected α-aminoacyl and peptidyl derivatives of cyanamide as prodrug forms of this alcohol deterrent agent.[9] Many of these prodrugs of cyanamide were highly potent, and some were long-acting. For example, stearoyl and palmitoyl cyanamide retained AlDH inhibitory activity for at least 72 h in rats and were equally effective by the oral route as well as by intraperitoneal administration. The activity of these cyanamide prodrugs is presumably mediated by enzymatic cleavage of the acyl cyanamide bond by endogenous hydrolytic enzymes to liberate cyanamide, which then inhibits AlDH after further bioactivation according to Scheme 1.

Scheme 1



We now describe a new series of novel, prodrug forms of cyanamide that require bioactivation by the hepatic cytochrome P-450 enzymes to release cyanamide in vivo. These cyanamide prodrugs were found to raise blood acetaldehyde (AcH) levels in rats following ethanol administration, a pharmacological consequence of the inhibition of hepatic AlDH.

Rationale

The report by Hanzlik et al.[10] that thiobenzamide (**1**) was oxidized by rat liver microsomes to give thiobenzamide *S*-dioxide (**2**) which spontaneously decomposed to benzonitrile (**3**) (Scheme 2) suggested that oxidation of thiourea itself by the same enzyme system might lead to the formation of cyanamide by an analogous mechanism (not shown). However, thiourea is known to be toxic and is also a suspect carcinogen and mutagen;[11] accordingly, it could not be considered further for potential drug development. On the other hand, to our knowledge, the oxidative metabolic fate of *S*-alkylisothioureas has not yet been described.

Thiobenzamide S-oxidation has been shown to be mediated in large part by the cytochrome P-450 mixed

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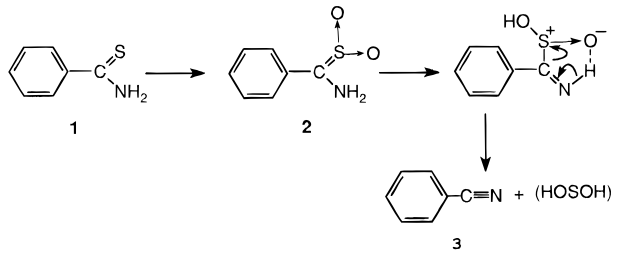
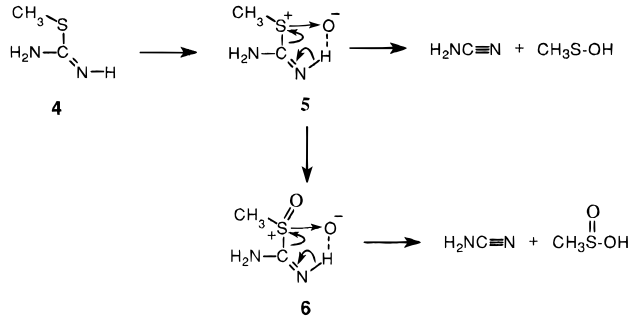
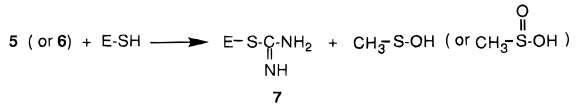
Scheme 2



Scheme 3



Scheme 4



function oxidase system,[10b] although the participation of flavin-containing monooxygenases cannot be discounted.[12] Enzymatic oxidations on divalent sulfur are known to yield sulfoxides and, ultimately, *S*-dioxides (or sulfones from dialkyl, diaryl, or alkylaryl sulfides).[13] Accordingly *S*-methyisothiourea (**4**) would be expected to be oxidized in vivo to its *S*-oxide (**5**) or the *S*-dioxide (or sulfone equivalent) **6**. Intermediates **5** or **6**, or both, should then spontaneously decompose to cyanamide and methanesulfenic acid or methanesulfinic acid, respectively, according to Scheme 3.

Alternatively, the S-oxidized **5** or **6** may react directly with the active site sulfhydryl group (Cys-302) of AlDH by carboxamidinating the enzyme (Scheme 4). The altered enzyme (**7**) will have a structure identical to that formed by reaction of cyanamide itself on the enzyme. This alternative mechanism bears a formal analogy to the action of the putative reactive metabolite of disulfiram, *viz.*, methyl *N*,*N*-diethylthiolcarbamate *S*-oxide (DETC-MeSO), which inactivates AlDH2 by carbamoylating the active-site sulfhydryl group.[14]

S-Methylisothiourea (**4**) would, therefore, be expected to behave as a prodrug of cyanamide in vivo in the manner depicted in Scheme 3 and to inhibit AlDH following further bioactivation to nitroxyl (Scheme 1) or, alternatively, may inhibit the enzyme directly following the initial bioactivation step (Scheme 4). Thus, administration of **4** to rats followed by an ethanol dose should give rise to elevated blood AcH levels, reflecting the inhibition of hepatic AlDH (by >70%[15]). The corresponding oxygen analog, *O*-methylisourea (structure not shown), which cannot be oxidatively metabolized in the manner depicted in Scheme 3, should have no effect on AlDH in vivo, being likely to be O-demethylated to urea and formaldehyde.

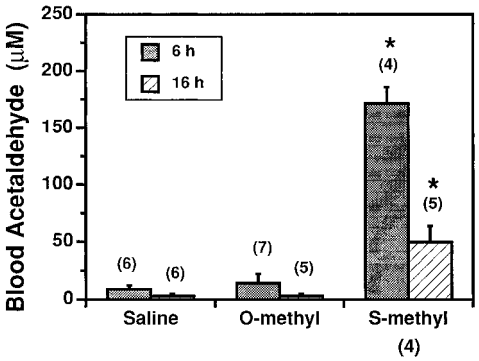
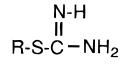


Figure 1. Effect of *S*-methylisothiourea (**4**) and *O*-methylisourea on ethanol-derived blood acetaldehyde levels in male Sprague−Dawley rats. Results are expressed as means ± SEM; the *N*'s are indicated in (parentheses) over the bars. The description of the drug administration protocols can be found in the Experimental Section. An asterisk (*) means significantly different from saline control and the O-methyl compound ($P < 0.05$).

Chart 1



Cpd No.	R
4	CH_3
8	n-C_4H_9
9	i-C_4H_9
10	CH_2=$CHCH_2$
11	$C_6H_5CH_2$
12	n-$C_{16}H_{33}$
13	$CH_3CONHCH_2CH_2$

Results

Administration of the commercially available **4** to rats (0.5 mmol/kg, ip) followed by an ethanol challenge (2.0 g/kg, ip) 5 h later, and measurement of blood AcH 1 h post ethanol, gave rise to a 119-fold increase in blood AcH levels compared to saline controls given ethanol (Figure 1). Blood AcH still persisted above saline control levels even at 16 h post drug treatment. As predicted, blood AcH was *not* elevated above control levels when *O*-methylisourea was administered to rats using the same protocol.

Other *S*-alkylisothioureas evaluated for their potential to oxidatively liberate cyanamide in vivo are listed in Chart 1. With the exception of compound **10**, all of these S-substituted isothioureas are known, and literature procedures were used or adapted for their preparation. Compound **13** was prepared by S-alkylation of thiourea with *N*-(2-chloroethyl)acetamide.[16]

Structural alteration of the *S*-alkyl group, i.e., replacement of the *S*-methyl group with *S*-*n*-butyl (**8**), *S*-isobutyl (**9**), or *S*-allyl (**10**) groups, resulted in increased potency in inhibiting hepatic AlDH, as reflected by ethanol-derived blood AcH levels that were 40−92% higher than that observed for **4** (Figure 2). The longer duration of action of **8** and **9** compared to cyanamide was evidenced by the persistently elevated blood AcH levels when measured 1 h following an ethanol challenge at 15 h post drug treatment. It is noteworthy that blood ethanol levels were unaffected by these structural modifications of **4** and were statistically not different from the blood ethanol of the saline control group (Figure 3, Supporting Information), suggesting that the

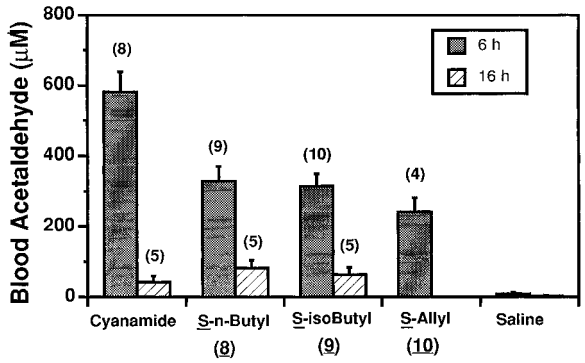


Figure 2. Ethanol-derived blood AcH in rats following pretreatment with the S-substituted *n*-butyl (**8**), isobutyl (**9**), and allyl (**10**) isothioureas compared to cyanamide and saline controls at 6 and 16 h post drug treatment. Statistical treatment as in Figure 1 legend. All values were significantly different ($P < 0.05$) from the saline controls.

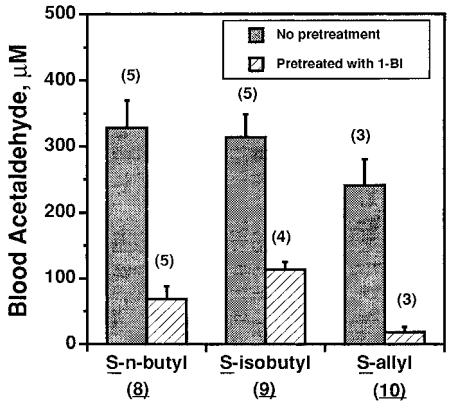


Figure 4. Effect of pretreatment with 1-benzylimidazole (0.03 mmol/kg, ip) on ethanol-derived blood AcH levels following the administration of *S*-alkylisothioureas (0.5mmol/kg, ip) to rats. The inhibitor was given 0.5 h prior to the *S*-alkylisothiourea and blood AcH levels were measured 1 h following the ethanol challenge. Statistical treatment as in Figure 1 legend.

first step in ethanol metabolism, *viz.*, conversion of ethanol to AcH, was not affected.

That the hepatic microsomal mixed function oxidase enzyme(s) were involved in the biotransformation of **8**, **9**, or **10** with subsequent inhibition of AlDH was adduced by the dramatic reduction in ethanol-derived blood AcH levels following the administration of **8**, **9**, or **10** (82, 66, and 88% reduction, respectively) to rats pretreated with 1-benzylimidazole (0.03 mmol/kg, ip), a known inhibitor of cytochrome P-450[17] (Figure 4). In further support, when the *n*-butyl analog **8** was incubated with rat liver microsomes coupled to catalase and yeast AlDH, the absolute requirement for microsomes and the NADPH-generating system was clearly indicated for the inhibition of AlDH (Table 1, Supporting Information). In contrast to cyanamide itself which served as positive control in this system, added catalase only partially enhanced the inhibition of AlDH by **8** (10 mM, Figure 5). However, since isolated liver microsomes invariably contain catalase,[7] addition of catalase was not absolutely required for AlDH inhibition by cyanamide.

The actual formation of cyanamide by cytochrome P-450 action on **8** was demonstrated by incubation of **8** with liver microsomes isolated from rats pretreated with 3-amino-1,2,4-triazole (to inhibit catalase) in the presence and absence of the NADPH-generating system,

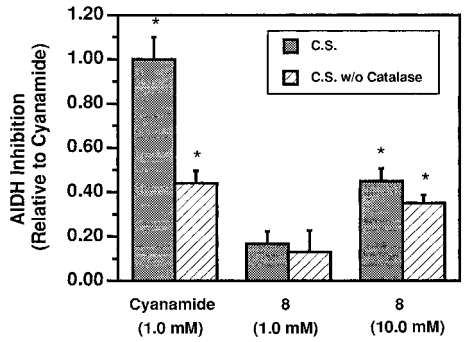


Figure 5. Effect of added catalase on the microsomal bioactivation of **8** to an inhibitor of yeast AlDH in vitro. C.S. = complete system. The details of the experiment are described in the Experimental Section. The inhibition observed with 1.0 mM cyanamide (positive control) in the C.S. was set at 1.0. An askerisk (*) means significantly different from control, i.e., without inhibitor ($P < 0.05$).

NADP + glucose + glucose 6-phosphate dehydrogenase. The cyanamide produced was extracted into ethyl acetate and derivatized by dansylation. The dansylated cyanamide was then separated by thin layer chromatography and the fluorescent product visualized and compared to an authenic sample of dansylated cyanamide (Figure 7, Supporting Information). Cyanamide formation was minimal in the absence of the NADPH-generating system, indicating the enzymatic nature of this reaction. Moveover, microsomes preincubated in the absence of cofactor still appeared to be fully capable of producing cyanamide (Figure 7, Supplementary Information), suggesting that flavin monoxygenase was not involved in the sulfoxidation of these *S*-alkylisothioureas.

Toxicity of Higher Homologs

On the basis of these favorable in vitro and in vivo results and the apparent absence of toxicity seen with the above compounds, we introduced a benzyl side chain as well as a long aliphatic side chain as the S-substitutent on the isothiourea in anticipation that such lipophilic groups would greatly prolong activity. These compounds are represented by the hydrochloride and hydrobromide salts of *S*-benzylisothiourea (**11**) and *S*-*n*-hexadecylisothiourea (**12**) (Chart 1), respectively. Surprisingly, when the *S*-benzyl compound (**11**) was administered to rats at a dose of 0.5 mmol/kg, ip, all rats ($n = 3$) died even before ethanol could be administered at 5 h. Lowering the dose to 0.10 mmol/kg, ip, allowed survival of the animals, but blood AcH levels were not statistically different from controls (data not shown). The *S*-*n*-hexadecyl analog (**12**) at 0.50 mmol/kg, ip, did give rise to elevated blood AcH when measured at 6 h post drug and 1 h post ethanol administration (Figure 6), but postmortem examination of the liver and kidneys at sacrifice showed extensive necrosis. Reducing the dose to 0.25 mmol/kg, ip, did not appreciably alleviate the liver and kidney damage observed at the higher dose. Although the elevated blood AcH levels measured at 16 h following this lower dose of **12** are not comparable to the results with the higher dose measured at 6 h (Figure 6), their higher levels relative to saline controls were unequivocal ($P < 0.05$).

In order to introduce a biocompatible S-substituted side chain, the *N*-acetyl derivative (**13**) of *S*-(2-aminoethyl)isothiourea (AET) was prepared, the latter, a

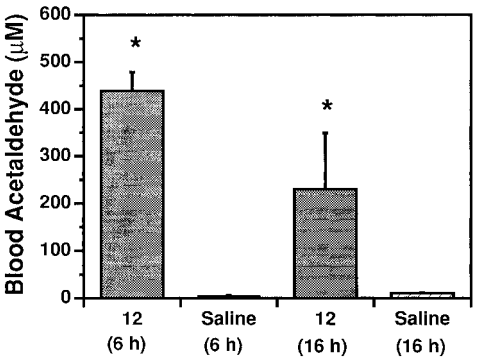


Figure 6. Ethanol-derived blood AcH in rats following treatment with *S*-hexadecylisothiourea (**12**), 0.5 mmol/kg (6 h), and 0.25 mmol/kg (16 h); $N = 3$ for **12** and $N = 5$ for the saline controls ($P < 0.05$).

compound that protects mice against lethal doses of *x*-radiation[18] possibly by intramolecular rearrangement to (mercaptoethyl)guanidine.[16] Administration of compound **13** to rats elevated blood AcH levels following ethanol administration significantly above the control levels without evidence of toxicity; however, potency (blood AcH = 67.0 ± 17.4 μM; blood EtOH = 50.1 ± 1.5 mM; $n = 3$) was only approximately one-third that observed for **4** itself.

Discussion

S-Methylisothiourea (**4**), the lowest member of this series, has been reported to cause a prompt rise in arterial pressure and to elicit bradycardia and increased response to the pressor action of adrenaline on intravenous administration to dogs, cats, and rabbits.[19a] Compound **4** has also been reported to have therapeutic efficacy in the treatment of septic shock elicited experimentally in rodents with bacterial lipopolysaccharide (endotoxin).[20] *S*-Alkylisothioureas also inhibit amide oxidase activity.[21] However, we are unaware of any report of its disulfiram-like adverse reaction with ethanol. The present study constitutes the first observation that *S*-alkylisothioureas can be oxidized by the hepatic mixed function oxidase system both in vivo and in vitro to (presumably) an *S*-oxide or an *S*-dioxide, which spontaneously releases cyanamide, a potent inhibitor of AlDH. However, direct inhibition of the enzyme by the sulfoxidized intermediate by carboxamidination of the active-site sulfhydryl group is also possible (Scheme 4). The in vitro results (Figure 5 and Table 1, Supplementary Information) where added catalase only slightly increased the inhibitory potency of microsome-activated compound **8** suggest that this alternative mechanism is also viable. However, no distinction between the two mechanisms can be made at this juncture since cyanamide was unequivocally identified as a product of the microsomal metabolism of **8** (Figure 7, Supplementary Information).

The toxicity of *S*-benzylisothiourea (**11**) was unexpected, but we suggest that this may be elicited by liberating thiourea, a known chemical toxin, by preferential benzylic oxidation as shown in Scheme 5 (Supporting Information) or, alternatively, by *para* hydroxylation followed by elimination. Thus, compound **11** may be considered to be a *pro* form of thiourea itself instead of a prodrug of cyanamide. The hepatic and renal toxicity shown by the hexadecyl compound **12**, in addition to AlDH inhibition, may also reflect the finite release of thiourea following dealkylation of the *n*-hexadecyl group by alternative and regioselective side chain α-hydroxylation, a reaction complementary to *S*-oxidation, this being likely enhanced by the lipophilic long alkyl chain.

Recently, Garvey et al.[22] have reported that *S*-ethylisothiourea and a wide series of structurally related compounds were potent inhibitors of human nitric oxide synthetase (NOS) enzymes in vitro, including the inducible (i), endothelial (e), and neuronal (n) forms of the enzyme. Interestingly, the series included the *S*-methyl-(**4**), *S*-*n*-butyl- (**8**), *S*-isobutyl- (**9**), *S*-2-methylallyl- (related to our **10**), *S*-benzyl-(**11**), and *S*-(2-aminoethyl)- (related to **13**) isothioureas. Although a representative group including the *S*-ethyl compound, when tested in *whole cells*, were less effective inhibitors than when the isolated enzymes were used, none were tested in vivo. On the basis of the present studies, toxicities associated with compounds that can liberate thiourea by enzymatic *S*-dealkylation of the isothioureas may be expected, and caution must be exercised in extrapolating in vitro data with isolated enzymes to in vivo efficacy.

In summary, we have shown that short-chain ($<C_4$) *S*-alkylisothioureas can be considered to be prodrug forms of the alcohol deterrent agent, cyanamide, on the basis that these isothioureas are bioactivated both in vivo and in vitro by the hepatic mixed function oxidase enzymes to reactive products that may inactivate AlDH directly, or may in fact liberate cyanamide, which on further bioactivation can inhibit this enzyme, thereby giving rise to elevated ethanol-derived blood AcH. Larger alkyl-substituted isothioureas are toxic to the liver and kidneys, presumably by releasing thiourea itself, a known toxic substance.

Experimental Section

Melting points were taken either on a Mettler capillary melting point apparatus or on a Fischer-Johns hot stage melting point apparatus and are uncorrected. When melting point discrepancies with literature values were found, the compounds were submitted for elemental analysis. IR spectra were recorded on a Nicollet FT-IR spectrophotometer. ^{1}H NMR or ^{13}C NMR spectra were recorded on a Varian T-60A or GE Unity 300 spectrophotometer, respectively. Except where indicated, thin layer chromatography (TLC) was performed using silica gel plates containing a fluorescent indicator, and the spots corresponding to the compounds were visualized either by fluorescent quenching observed under UV light or by exposure to iodine vapors in an iodine chamber.

Reagents and Chemicals. The dihydrogen sulfate salts of *O*-methylisourea and *S*-methylisothiourea (**4**) were purchased from Fluka Chemical Co. (Ronkonkoma, NY) and Aldrich Chemical Co. (Milwaukee, WI), respectively. All other S-alkylated isothioureas were prepared according to or by adaptation of literature procedures. Glucose 6-phosphate, glucose 6-phosphate dehydrogenase, cyanamide, bovine liver catalase, and yeast AlDH were purchased from Sigma Chemical Co. (St. Louis, MO).

***S*-*n*-Butylisothiourea (8) Hydrobromide; *S*-Isobutylisothiourea (9) Hydrochloride; *S*-Benzylisothiourea (11) Hydrobromide.** These compounds were prepared according to literature procedures: **8**, mp 77−78 °C (lit.[23] mp 80−82 °C); **9**, mp 96−97 °C (lit. mp 96 °C,[23a] 103−105 °C[23b]); **11**, in two crops, mp 149−151 and 175−176 °C (lit.[23a] mp 146−148 and 176−177 °C); mp 112−113 °C.[23b] However, when the melting points were retaken 8 months later, both crops melted partially at ∼143 °C and fully at 152−153 °C. The NMR spectra of either crop in MeOH-d_4 were identical to each other, δ 7.39 (*m*, 5H) 4.46 (*s*, 2H). Anal. (either crop) ($C_8H_{11}N_2SCl$) C, H, N.

***S*-Allylisothiourea (10) Hydrochloride.** Obtained by heating allyl chloride (4.1 mL) and thiourea (3.80 g, 5.0 mmol) in absolute ethanol. Recrystallized from ethanol−ether (67% yield): mp 87−89 °C; ^{1}H NMR (D_2O) δ 5.80 (m, 1H), 5.26 (*m*, 2H) 3.65 (*d*, 2H, J = 6.5 Hz); ^{13}C NMR (D_2O) 170.6, 130.4, 119.8, 33.4 ppm. Anal. ($C_4H_9N_2SCl$) C, H, N.

***S*-*n*-Hexadecylisothiourea (12) Hydrobromide.** Recrystallized from ethanol−ether: mp 87−89 °C (lit.[24] mp 104−105 °C). Anal. ($C_{17}H_{37}NSBr$) C, H, N.

***S*-(Acetamidoethyl)isothiourea (13) Hydrochloride.**[16] Recrystallized from ethanol−ether: mp 196−198 °C (lit.[16] mp 193−194 °C).

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. This committee is vigorous in enforcing its charge of minimizing the use of animals in research.

Drug Administration Protocol. Sprague−Dawley male rats (Harlan Sprague−Dawley, Indianapolis, IN) weighing 170−220 g were fasted ~24 h prior to the time of sacrifice. All drugs were dissolved in isotonic saline except **12** which was suspended in 1% carboxymethylcellulose (CMC). Doses of 0.5 mmol/kg were given ip as 1.0 mL/100 g of body weight except where noted. Ethanol was administered 1 h before sacrifice and was given as a 20% (w/v) solution, 1.0 mL/100 g of body weight. The animals were sacrificed, and blood levels for AcH and ethanol measurements were determined as described below.

Measurement of Blood AcH and Ethanol Levels. Blood AcH and ethanol levels were measured 1 h after the administration of ethanol in treated and control animals as previously described.[9b]

In Vitro Studies. Microsomal Metabolism of 8 to an Inhibitor of AlDH. Liver microsomes were isolated from male, Sprague−Dawley rats after an overnight fast as described earlier.[15] The bioactivation of **8** to reactive metabolite(s) was estimated using a two-step assay system with the inhibition of yeast AlDH as the end point. The primary reaction mixture contained 100 mM potassium phosphate buffer (pH 7.5), 5.0 mM $MgCl_2$, 16 mM KCl, 2.5 mM glucose 6-phosphate, 0.1 μmol of $NADP^+$, and 1.0 unit of glucose 6-phosphate dehydrogenase. After a 3 min preincubation period, the reaction was initiated by the addition of 8.0 μg of catalase, 0.06 unit of yeast AlDH, the inhibitor **8**, or cyanamide where indicated, and 20 μL of liver microsomes (microsomal pellet reconstituted to 0.5 mL/g wet weight liver tissue with 0.15 M KCl). The total volume of the primary reaction mixture was 0.1 mL. This mixture was then incubated for 10 min at 37 °C. At 10 min, a 20 μL aliquot of the primary mixture was removed and added directly to a secondary reaction mixture containing 0.5 mM NAD^+, 1.0 mM EDTA, 30% glycerol, and 90 mM potassium phosphate buffer (pH 8.0) in a final volume of 1.0 mL. The reaction was initiated by the addition of benzaldehyde (0.6 μmol), and the remaining yeast AlDH activity in this secondary mixture was determined spectrophotometrically at 25 °C by following the increase in absorbance at 340 nm over time.

Microsomal Metabolism of 8 to Cyanamide. For this experiment, the rats were pretreated with 3-amino-1,2,4-triazole (12 mmol/kg) 1 h before sacrifice, and the livers were perfused with 0.15 M KCl to remove residual aminotriazole before isolation of the microsomes. Each incubation flask contained 100 mM potassium phosphate buffer, pH 7.4, 16.5 mM KCl, 4.00 mM $MgCl_2$, 50 mM **8**, microsomes from 4.0 g (wet wt) of liver, and, when indicated, the NADPH-generating system, *viz*., 2.0 mM NADP, 25 mM glucose 6-phosphate, 312 units of glucose 6-phosphate dehydrogenase, and water to a final volume of 10.0 mL. After incubation for 60 min at 37 °C, the microsomes were sedimented by centrifugation at 100000*g* for 60 min, and the supernatant fractions were processed for cyanamide determined essentially as described by Prunosa et al.,[25] except that the dansylation was allowed to proceed for 20 h at 40 °C. The product was separated by TLC on Macherney-Nagel silica gel (250 μm) plates without fluorescent background using EtOAc/MeOH/HOAc (100:5:5) as solvent, and the plates were viewed under 365 nm UV light.

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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Supporting Information Available: Table 1, Figures 3 and 7, and Scheme 5 (4 pages). Ordering information is given on any current masthead page.

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