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Synthesis of 1-Hydroxyalkyl-3-Substituted Ureas and Thioureas, Substrates for Alcohol Dehydrogenase

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Abstract—A series of 1-(2-hydroxyethyl)- and 1-(3-hydroxyethyl)-3-substituted ureas and thioureas were synthesized. 1-(3-Hydroxyethyl)-3-acylthioureas were shown to be specific substrates for alcohol dehydrogenase *in vitro.*

Key words: alcohol dehydrogenase, substrates; thiourea, 1,3-disubstituted; urea, 1,3-disubstituted

INTRODUCTION

Despite the medical achievements in the treatment of chronic alcoholism, the assortment of medicines for its correction is limited [1, 2].[2] Therefore, the search for the compounds affecting the ethanol biotransformation remains topical.

In this work, 1-hydroxyalkyl-3-substituted ureas and thioureas, which contain a primary hydroxyl group and structurally resemble the drug disulfiram (teturamin) [2, 3], became the subjects under study.

RESULTS AND DISCUSSION

Ureas (**I**)–(**VII**) and thioureas (**VIII**)–(**X**) were obtained by interaction of the corresponding iso(thio)cyanate with 2-aminoethanol and/or 3-aminopropanol as shown in Scheme 1. The synthesis of 1-(3-hydroxypropyl)-3-acylthioureas (**XI**) and (**XII**) is described in Scheme 2.

The kinetic parameters of the oxidation reaction of (**I**)–(**XII**) with a typical isoform of ADH from human liver were determined in experiments *in vitro* [4]. The ratio of the maximal oxidation rate (relative to ethanol, V_{rel}) to the Michaelis constant (K_m) was chosen as a parameter that, at low concentrations of the compounds tested, can characterize the substrate properties of the compounds and appeared to reflect the *in vivo* situation [5] (see table).

One can see from the table that the compounds under study tend to increase their substrate specificities to ADH when proceeding from hydroxyethylurea derivatives (**I**)–(**III**) to hydroxypropylurea derivatives (**IV**)–(**XII**) and from ureas (**I**)–(**VII**) to thioureas (**VIII**)–(**X**) and, further, to acylthioureas (**XI**) and (**XII**). The properties of 1-(3-hydroxypropyl)-3-(4-fluorobenzoyl)thiourea (**XII**) are of particular importance.

Thus, our results suggest that a further search for highly specific ADH substrates in the series of 1-(3-hydroxypropyl)-3-acylthioureas is promising, and these compounds might be used for the design of new medicines with the corresponding activity profile.

EXPERIMENTAL

The following reagents were used: 2-aminoethanol, 3-aminopropanol, benzoyl chloride, and 4-fluorobenzoyl chloride from Merck (Germany); *n*-butyl, *tert*-butyl, 3,4-dichlorophenyl, and phenyl isocyanates and ethyl, 4-fluorophenyl, and 2,5-xylyl isothiocyanates from Fluka (Switzerland). Other reagents and solvents were of domestic production.

Melting points were determined on a Mettler FP62 device (Switzerland).

^{1}H NMR spectra were obtained on a Bruker WD-80SY (Germany) spectrometer at the working frequency of 80 MHz in DMSO-d_6 (Fluka, Switzerland). Chemical shifts of protons were measured relative to the residual signal of solvent (δ 2.49 ppm) and are given in δ scale.

For the quantitative elemental analysis, a Carlo Erba 1106 CHN analyzer (Italy) was used.

Spectrophotometric measurements were performed on a Hitachi 557 instrument (Japan).

1-(2-Hydroxyethyl)-3-*n*-butylurea (I). *n*-Butyl isocyanate (3.50 g, 35.3 mmol) was added at vigorous stirring to a solution of 2-aminoethanol (2.16 g,

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[2] Abbreviations: ADH, alcohol dehydrogenase from human liver (alcohol:NAD$^+$ oxidoreductase, EC 1.1.1.1).

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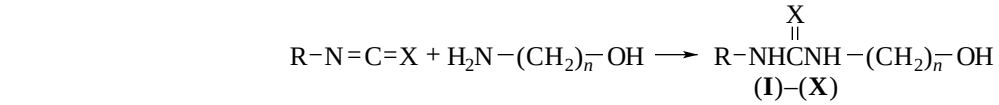


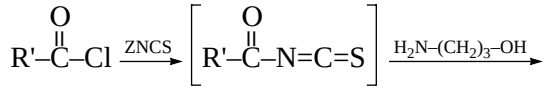
R	X	*n*		R	X	*n*	
n-C_4H_9	O	2	(**I**)	C_6H_5	O	3	(**VI**)
t-C_4H_9	O	2	(**II**)	3,4-$Cl_2C_6H_3$	O	3	(**VII**)
C_6H_5	O	2	(**III**)	C_2H_5	S	3	(**VIII**)
n-C_4H_9	O	3	(**IV**)	4-FC_6H_4	S	3	(**IX**)
t-C_4H_9	O	3	(**V**)	2,5-$(CH_3)_2C_6H_3$	S	3	(**X**)

Scheme 1.

35.4 mmol) in dry ether (15 ml). The reaction mixture was stirred for 1 h, and urea (**I**) was filtered and recrystallized from 95% ethanol; yield 5.12 g (91%); mp 66–67°C (ethanol); ^{1}H NMR: 0.90 (3 H, t, CH_3), 1.40 (2 H, m, CH_2), 1.65 (4 H, m, 2 CH_2), and 3.40 (4 H, m, CH_2N + CH_2O). Found, %: C 52.54, H 10.17, N 17.19. Calculated for $C_7H_{16}N_2O_2$, %: C 52.48, H 10.07, N 17.48.

1-(2-Hydroxyethyl)-3-*tert*-butylurea (II) was similarly obtained from *tert*-butyl isocyanate (1.68 g, 16.9 mmol) and 2-aminoethanol (1.03 g, 16.9 mmol); amorphous; yield 2.50 g (93%); ^{1}H NMR: 1.30 (9 H, s, 3 CH_3), 3.15, and 3.65 (4 H, 2 m, CH_2N and CH_2O). Found, %: C 52.61, H 9.89, N 17.25. Calculated for $C_7H_{16}N_2O_2$, %: C 52.48, H 10.07, N 17.48.

1-(2-Hydroxyethyl)-3-phenylurea (III) was similarly obtained from phenyl isocyanate (8.50 g, 71.4 mmol) and 2-aminoethanol (4.36 g, 71.4 mmol); yield 11.92 g (93%); mp 123°C (ethanol); ^{1}H NMR: 3.60 (4 H, m, CH_2N and CH_2O) and 7.20 (5 H, m C_6H_5). Found, %: C 60.16, H 6.47, N 15.35. Calculated for $C_9H_{12}N_2O_2$, %: C 59.99, H 6.71, N 15.55.



R'	Z	
C_6H_5	NH_4	(**XI**)
4-FC_6H_4	Na	(**XII**)

Scheme 2.

1-(2-Hydroxypropyl)-3-*n*-butylurea (IV) was similarly obtained from *n*-butyl isocyanate (47.0 g, 47.4 mmol) and 3-aminopropanol (3.56 g, 47.4 mmol); yield 7.76 g (94%); mp 66–67°C (ethanol); ^{1}H NMR: 0.90 (3 H, t, CH_3), 1.40 (2 H, m, CH_2), 1.65 (4 H, m, 2 CH_2), and 3.15 and 3.60 (6 H, m, 2 CH_2N + CH_2O). Found, %: C 55.21, H 10.32, N 15.96. Calculated for $C_8H_{18}N_2O_2$, %: C 55.15, H 10.41, N 16.08.

1-(2-Hydroxypropyl)-3-*tert*-butylurea (V) was similarly obtained from *tert*-butyl isocyanate (2.00 g, 20.2 mmol) and 3-aminopropanol (0.80 g, 10.7 mmol); amorphous; yield 2.31 g (78%); ^{1}H NMR: 1.30 (9 H, s, 3 CH_3), 1.65 (2 H, m, CH_2), and 3.15 and 3.60 (4 H, 2t, CH_2N + CH_2O). Found, %: C 55.31, H 10.25, N 15.84. Calculated for $C_8H_{18}N_2O_2$, %: C 55.15, H 10.41, N 16.08.

1-(3-Hydroxypropyl)-3-phenylurea (VI) was similarly obtained from phenyl isocyanate (1.59 g, 13.2 mmol) and 3-aminopropanol (1.00 g, 13.3 mmol); yield 2.13 g (82%); mp 113–114°C (ethanol); ^{1}H NMR: 1.74 (2 H, m, CH_2), 3.30 and 3.62 (4 H, 2m, CH_2N + CH_2O), and 7.20 (5 H, m, C_6H_5). Found, %: C 62.03, H 7.12, N 14.28. Calculated for $C_{10}H_{14}N_2O_2$, %: C 61.84, H 7.27, N 14.42.

1-(3-Hydroxypropyl)-3-(3,4-dichlorophenyl)urea (VII) was similarly obtained from 3,4-dichlorophenyl isocyanate (2.00 g, 10.6 mmol) and 3-aminopropanol (0.80 g, 10.7 mmol); yield 1.65 g (59%); ^{1}H NMR: 1.75 (2 H, m, CH_2), 3.25 and 3.65 (4 H, 2m, CH_2N + CH_2O), and 7.50 (3 H, m, C_6H_3). Found, %: C 45.93, H 4.51, N 10.44. Calculated for $C_{10}H_{12}Cl_2N_2O_2$, %: C 45.65, H 4.60, N 10.65.

1-(3-Hydroxypropyl)-3-ethylthiourea (VIII) was similarly obtained from ethyl isothiocyanate (2.00 g, 23.0 mmol) and 3-aminopropanol (1.78 g, 23.7 mmol); yield 3.21 g (86%); mp 86–87°C (ethanol); ^{1}H NMR: 1.15 (3 H, t, CH_3), 1.75 (2 H, m, CH_2), and 3.55 (4 H, m, CH_2N + CH_2O). Found, %: C 44.51, H 8.80, N

Kinetic parameters of the oxidation reaction of 1,3-disubstituted (thio)ureas catalyzed by ADH

Compound	Concentration range, mM	V_{rel}	K_m, mM	V_{rel}/K_m
(**I**)	0.04–0.6	0.08	0.21	0.38
(**II**)	0.04–0.6	0.07	0.27	0.26
(**III**)	0.1–1.4	0.11	0.46	0.24
(**IV**)	0.04–0.6	0.12	0.16	0.75
(**V**)	0.5–2.4	0.19	1.18	0.16
(**VI**)	0.05–0.3	0.16	0.10	1.60
(**VII**)	0.1–1.6	0.23	0.52	0.44
(**VIII**)	0.4–4.1	0.18	1.37	0.13
(**IX**)	0.04–0.4	0.23	0.07	3.30
(**X**)	0.1–0.5	0.18	0.20	0.90
(**XI**)	0.1–0.5	0.79	0.21	3.76
(**XII**)	0.1–1.6	2.71	0.54	5.00

17.67. Calculated for $C_6H_{14}N_2OS$, %: C 44.41, H 8.70, N 17.26.

1-(3-Hydroxypropyl)-3-(4-fluorophenyl)thiourea (IX) was similarly obtained from 4-fluorophenyl isothiocyanate (2.50 g, 16.3 mmol) and 3-aminopropanol (1.22 g, 16.2 mmol); yield 3.20 g (86%); mp 112–113°C (ethanol); ^{1}H NMR: 1.80 (2 H, m, CH_2), 3.80 (4 H, m, $CH_2N + CH_2$), and 7.15 (4 H, m, C_6H_4). Found, %: C 52.90, H 5.63, N 12.13. Calculated for $C_{10}H_{13}FN_2OS$, %: C 52.61, H 5.74, N 12.27.

1-(3-Hydroxypropyl)-3-(2,5-xylyl)thiourea (X) was similarly obtained from 2,5-xylyl isothiocyanate (1.09 g, 6.68 mmol) and 3-aminopropanol (0.50 g, 6.66 mmol); yield 1.07 g (67%); mp 97°C (ethanol); ^{1}H NMR: 1.75 (2 H, m, CH_2), 2.20 and 2.30 (6 H, 2 s, 2 CH_3), 4.60 (4 H, m, $CH_2N + CH_2O$), and 7.05 (3 H, m, C_6H_3). Found, %: C 60.29, H 7.67, N 11.49. Calculated for $C_{12}H_{18}N_2OS$, %: C 60.47, H 7.61, N 11.75.

1-(3-Hydroxypropyl)-3-benzoylthiourea (XI). Benzoyl chloride (14.06 g, 100 mmol) was added to a suspension of ammonium rhodanide (7.61 g, 100 mmol) in hot anhydrous acetone (25 ml). The mixture was refluxed for 5 min, cooled to room temperature, and filtered. The filtrate was added dropwise to a solution of 3-aminopropanol (7.51 g, 100 mmol) in acetone (25 ml); the mixture was mixed for 1 h and then poured into 10 vols of water. The precipitate of (**XI**) was separated and recrystallized from aqueous acetone to give (**XI**); yield 17.60 g (74%); mp 83–84°C (acetone–water); ^{1}H NMR: 1.90 (2 H, m, CH_2), 3.70 (4 H, m, $CH_2N + CH_2O$), and 7.70 (5 H, m, C_6H_5). Found, %: C 56.00, H 6.23, N 11.43. Calculated for $C_{11}H_{14}N_2O_2S$, %: C 55.44, H 5.92, N 11.76.

1-(3-Hydroxypropyl)-3-(4-fluorobenzoyl)thiourea (XII) was similarly obtained from sodium thiocyanate (3.12 g, 38.5 mmol), 4-fluorobenzoyl chloride (6.10 g, 38.5 mmol) and 3-aminopropanol (2.90 g, 38.6 mmol); yield 4.31 g (44%); mp 85–86°C (acetone–water); ^{1}H NMR: 1.90 (2 H, m, CH_2), 3.80 (4 H, m, $CH_2N + CH_2O$), and 7.50 (4 H, m, C_6H_4). Found, %: C 51.13, H 5.08, N 10.54. Calculated for $C_{11}H_{13}FN_2O_2S$, %: C 51.55, H 5.11, N 10.93.

Substrate properties of (I)–(XII) toward ADH. Alcohol dehydrogenase was isolated from a homogenate of the human liver as described in [6]. The homogenate was centrifuged at 30000 *g* for 10 min, and the supernatant was once more centrifuged at 105000 *g* for 120 min and lyophilized. The residue was dissolved in 20 mM Tris-HCl buffer, pH 8.2, and applied onto a column (2.3 × 50 cm) packed with DEAE cellulose equilibrated in the same buffer. The column was eluted with a linear gradient of Tris-HCl (pH 7.4) from 0 to 1 M, and the fraction containing ADH (monitoring at 280 nm and by the dehydrogenase activity, eluted within the concentration range 0.46–0.52 M) was collected and lyophilized up to the final protein concentration of 1–2 mg/ml. The preparation was stored at –80°C.

The ADH activity was spectrophotometrically determined according to the increase in the optical density at 340 nm. The reaction was initiated by addition of ethanol or one of (**I**)–(**XII**), dissolved in DMSO to final concentration given in the table, to a solution (3 ml) containing 0.1 mg/ml ADH and saturating concentration of NAD^+ (3×10^{-3} M) in 50 mM pyrophosphate buffer (pH 7.4). To calculate the amount of NADH formed, the molar extinction coefficient $\varepsilon = 6210\ M^{-1}\ cm^{-1}$ was used. The values of K_m and V_{rel} were determined graphically in the Lineweaver–Burk coordinates [7].

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