Inhibition of Human Alcohol Dehydrogenases by Formamides

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Human alcohol dehydrogenase (*Hs*ADH) comprises class I (α, *β*, and *γ*), class II (*π*), and class IV (*σ*) enzymes. Selective inhibitors of the enzymes could be used to prevent the metabolism of alcohols that form toxic products. Formamides are unreactive analogues of aldehydes and bind to the enzyme−NADH complex [Ramaswamy, S.; Scholze, M.; Plapp, B. V. *Biochemistry* **1997**, *36*, 3522−3527]. They are uncompetitive inhibitors against varied concentrations of alcohol, and this makes them effective even with saturating concentrations of alcohols. Molecular modeling led to the design and synthesis of a series of cyclic, linear, and disubstituted formamides. Evaluation of 23 compounds provided structure−function information and selective inhibitors for the enzymes, which have overlapping but differing substrate specificities. Monosubstituted formamides are good inhibitors of class I and II enzymes, and disubstituted formamides are selective for the α enzyme. Selective inhibitors, with K_i values at pH 7 and 25 °C of 0.33−0.74 μM, include *N*-cyclopentyl-*N*-cyclobutylformamide for *Hs*ADH α, *N*-benzylformamide for *Hs*ADH β_1, *N*-1-methylheptylformamide for *Hs*ADH γ_2, and *N*-heptylformamide for *Hs*ADH *σ* and *Hs*ADH β_1.

Introduction

Human alcohol dehydrogenases (*Hs*ADH[1]) are targets for inhibitor design since they oxidize alcohols to toxic products and they can participate in the altered metabolism of various compounds during ethanol consumption.[2,3] There are five enzymes with significant activity on a variety of alcohols: class I (α, *β*, and *γ*), class II (*π*), and class IV (*σ*). Selective, uncompetitive inhibitors could be especially useful for controlling the metabolism of alcohols since they are effective in the presence of saturating concentrations of alcohols.[4] Formamides and amides bind preferentially to the enzyme−NADH complex and are potent uncompetitive inhibitors against varied concentrations of ethanol.[5−8]

The design of inhibitors for the human enzymes can be based on inhibitors of horse liver alcohol dehydrogenase (*Eq*ADH) due to the structural homology among these enzymes.[9,10] We used the three-dimensional structure of *Eq*ADH complexed with NADH and *N*-cyclohexylformamide to provide a starting model.[8] Three-dimensional structures of *β* and *σ* enzymes are known,[9,10] and models of the α, γ_2, and *π* enzymes were created by making the appropriate amino acid substitutions in the structure of *EqADH* (Table 1). Since the structures of the active sites are different, we expected to be able to produce selective inhibitors.

Table 1. Active-Site Residues of Alcohol Dehydrogenases[11,12]

amino acid	*Eq*	*Hs* α	*Hs* β_1	*Hs* γ_2	*Hs* *π*	*Hs* *σ*
48	S	T	T	S	T	T
57	L	M	L	L	F	M
93	F	A	F	F	Y	F
110	F	Y	Y	Y	L	L
116	L	V	L	L	L	I
117	S	S	G	G	S	Δ[a]
140	F	F	F	F	F	F
141	L	L	L	V	F	M
143	T	I	T	V	T	T
294	V	V	V	V	V	V
306[b]	M	M	M	M	E	M
309[b]	L	L	L	L	I	F
318	I	I	V	I	F	V

[a] Amino acid residue 117 in the human *σ* enzyme is deleted. [b] Residues provided by the second subunit.

Results

Enzymology. The inhibition constants were determined by initial velocity studies with varied concentrations of the inhibitor and a substrate appropriate for each human enzyme. The K_i values were not dependent on the substrate used. The formamides are uncompetitive inhibitors against alcohols and competitive against aldehydes or ketones, indicating that the formamides bind to the enzyme−NADH complex. The inhibition constants determined for the forward or reverse reaction were about (within 2-fold) the same, which is expected if coenzyme dissociation is rate-limiting in the ordered mechanism. Most values were determined at pH 7, but similar values were also obtained at pH 8. Inhibition of *Eq*ADH by *N*-cyclohexylformamide is independent of pH in the range 5.5−9.0 and decreases above a p*K* of 10.5.

Structure−Function Relationships. Table 2 shows that the binding affinities of *Eq*ADH, α, and β_1 enzymes for the cyclic formamides are independent of ring size (excluding cyclopropyl). Each of these enzymes has exceptionally high affinities for the *N*-cyclobutyl, *N*-cyclopentyl, and *N*-cyclohexyl derivatives. In contrast, the γ_2 and *π* enzymes show an increase in affinity as the ring size is increased. The class I enzymes have similar affinity for *N*-cyclohexylformamide, whereas the *σ* and *π* enzymes have much lower affinity for this inhibitor. The binding affinity of *Hs*ADH *σ* also shows little dependence on ring size, but there was decreased affinity for *N*-cyclohexylformamide, which implicates

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Table 2. Inhibition of Alcohol Dehydrogenases by Formamides

		K_i (μM)					
R_1	R_2	*Eq*E[a]	*Hs* α[b]	*Hs* β_1[c]	Hs γ_2[d]	*Hs* π[e]	*Hs* σ[f]
cyclopropyl	H	100	33	6.4	700	5200	300
cyclobutyl	H	1.7	3.8	2.9	45	450	54
cyclopentyl	H	7.6	3.9	7.3	13	150	28
cyclohexyl	H	8.7	2.3	3.4	5.2	84	380
cyclohexylmethyl	H	12	3.6	1.0	5.8	7.1	47
benzyl	H	0.74	31	**0.33**	4.9	110	11
n-propyl	H	10[g]	5.6[h]	5.5	120	300	21
isopropyl	H	2.6	22	19	15	1900	210
n-butyl	H	16	7.2	11	100	110	30
isobutyl	H	13	5.5	26	54	340	180
n-heptyl	H	3.0	3.6	**0.33**	12	11	**0.74**
1-methylheptyl	H	5.4	7.0	1.7	**0.41**	40	100
cyclopropyl	heptyl	1100	1.9	1900	2.1	31	960
cyclobutyl	cyclobutyl	7200	1.8	4900	19	280	1800
cyclopentyl	cyclopropyl	110	0.88	4600	**4.6**	96	930
cyclopentyl	cyclobutyl	8600	**0.36**	10000	47	360	1100
cyclopentyl	cyclopentyl	2600	7.4	1600	1000	ND	ND
cyclopentyl	propyl	1900	4.5	2800	21	191	1700
cyclohexyl	methyl	600	5.5	920	40	120	4900
cyclohexyl	ethyl	1400	4.5	1500	33	ND	ND
cyclohexyl	propyl	5200	14	4000	160	ND	ND
cyclohexyl	isopropyl	470	24	46	83	150	1300
cyclohexyl	cyclopropyl	440	5.9	250	120	ND	640

[a] Inhibition constants determined with ethanol (0.4−2.0 mM) as the varied substrate, pH 8.0. [b] Ethanol (2.7−15 mM), pH 8.0. [c] Acetaldehyde (0.02−0.50 mM), pH 7.0. [d] Cyclohexanone (1.5−12 mM), pH 7.0. [e] Acetaldehyde (5−50 mM), pH 7.0. [f] Ethanol (30−150 mM), pH 7.0. [g] Rest of column with cyclohexanone (10−50 mM), pH 7.0. [h] Rest of column with cyclohexanone (0.069−0.96 mM), pH 7.0. ND = not determined. The inhibition constants had errors ≤15%.

steric effects. The cyclohexylmethyl derivative was a good inhibitor for all enzymes except for *Hs*ADH σ.

The substitution of the R_1 position with a phenyl group resulted in a very high K_i value for *Eq*ADH (2.5 mM). In contrast, *N*-benzylformamide binds tightly to *Eq*ADH[7] and was the most potent and selective inhibitor for *Hs*ADH β_1 (K_i = 0.33 μM). The π enzyme, which has good affinity for *N*-cyclohexylmethylformamide, was much less sensitive to the *N*-benzyl derivative.

The affinities of the horse, α, β_1, and σ enzymes for the noncyclic, linear formamides showed little dependence on the length of the alkyl chain. This is a somewhat surprising result, as the substrate binding pockets are completely hydrophobic and each methylene unit can be expected to improve affinity by about 2-fold.[5] However, *Hs*ADH β_1 and *Hs*ADH σ showed particularly strong affinities for *N*-heptylformamide, which indicates that a certain size of aliphatic chain can optimize interactions. It is interesting that the *Hs*ADH γ_2 prefers the branched aliphatic chain over the corresponding linear chain, whereas the π and σ enzymes prefer the linear chains. The affinity of the γ_2 enzyme for the branched 1-methylheptyl derivative is particularly strong, which must reflect the space near the catalytic zinc. The β_1 enzyme also had a high affinity for the 1-methylheptyl derivative, whereas *Hs*ADH σ was poorly inhibited.

We examined a series of disubstituted formamides as inhibitors since modeling suggested that the α enzyme should have increased space near the catalytic zinc due to the replacement of Phe-93 with Ala. Indeed, the whole series of compounds with substituents at the R_2 position showed high affinities for the α enzyme. In particular, *N*-cyclopentyl-*N*-cyclobutylformamide was an especially potent inhibitor of *Hs*ADH α. In contrast, the horse, σ, β_1, and π enzymes have much lower affinities for these compounds than they do for the monosubstituted derivatives. Surprisingly, the γ_2 enzyme binds the disubstituted derivatives about as well as the monosubstituted compounds. This result indicates again that the space near the catalytic zinc of the γ_2 enzyme is larger than predicted from the modeling studies.

Discussion

The three-dimensional structure of the *Eq*ADH−NADH−*N*-cyclohexylformamide complex suggests that the amide resembles the ground-state structure for aldehyde substrates, as the oxygen is ligated to the catalytic zinc and the carbonyl carbon is suitably positioned for direct hydrogen transfer.[8] The inhibitor binds with a cis conformation, which produces a cation−π interaction between the amide N−H and the benzene ring of Phe-93 of the enzyme. We expected that the human enzymes would have different specificities for the various formamides, reflecting the different constellation of amino acid residues in the active sites and the flexibility of binding modes. This expectation was realized, and particularly selective inhibitors were identified. Since three-dimensional structures of the human enzymes complexed with the new formamides are not available, we must explain the structure−function relationships in terms of models based on known structures. We presume that tight binding is associated with good van der Waals contacts, hydrophobic interactions (and lack of voids that would accommodate water molecules), and the absence of steric conflicts. The binding modes will vary due to flexible side chains that adapt to the ligand structure and optimize interactions.

The disubstituted formamide *N*-cyclopentyl-*N*-cyclobutylformamide is a potent and selective inhibitor for

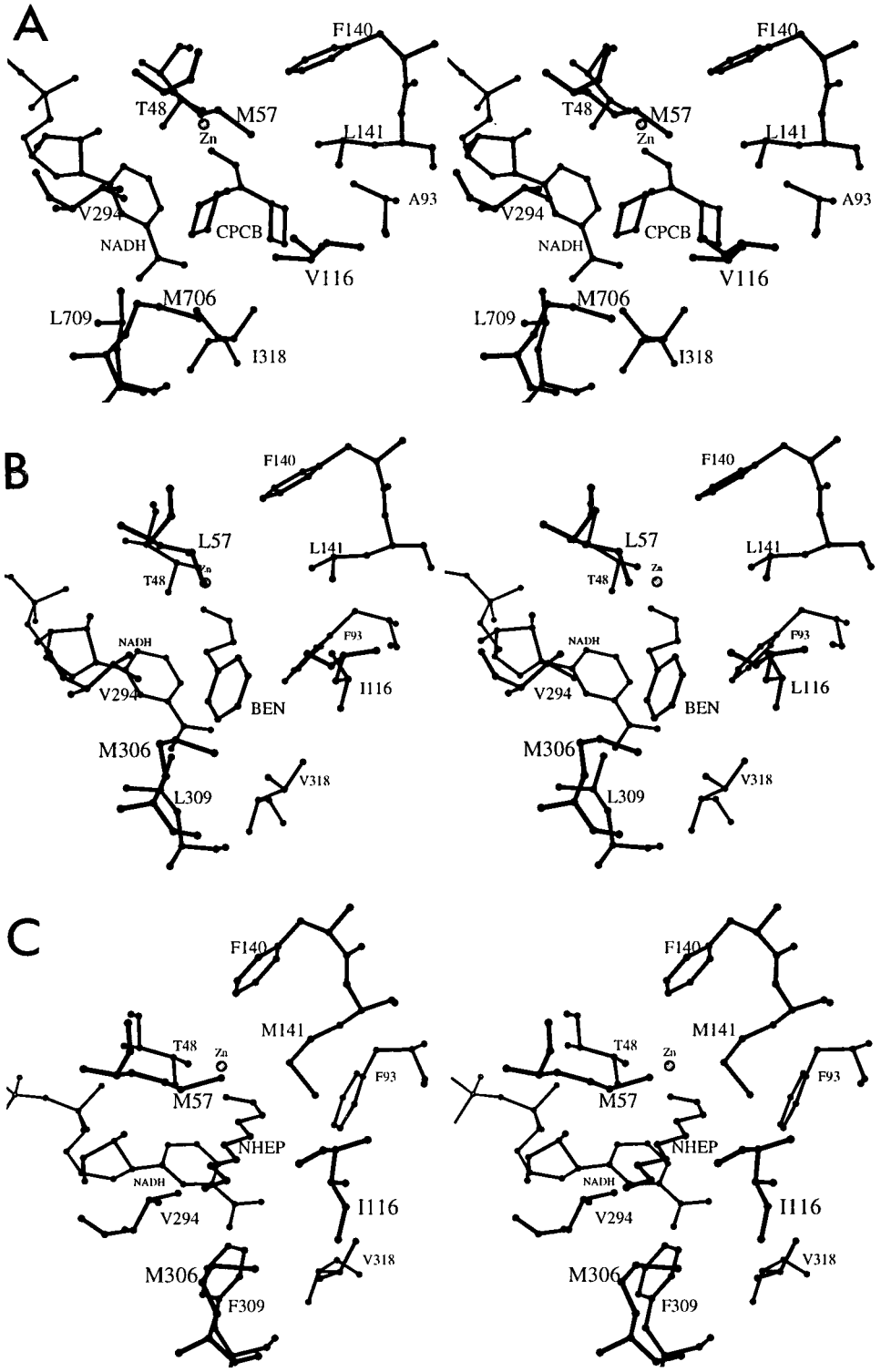

Figure 1. Models of the proposed binding modes of the formamides with the human enzymes. (A) The *Z* isomer of *N*-cyclopentyl-*N*-cyclobutylformamide binding to *Hs*ADH α. The model of the α enzyme was created by making the appropriate amino acid substitutions in the structure of *Eq*ADH. (B) The cis isomer of *N*-benzylformamide binding to *Hs*ADH β_1.[9] (C) The cis isomer of *N*-heptylformamide binding to *Hs*ADH σ.[10]

*Hs*ADH α. Molecular modeling suggests that this inhibitor binds to the α enzyme in the *Z* configuration (Figure 1A). This orientation produces favorable contacts between the cyclobutyl group and Ala-93 and Ile-318, whereas the *E* configuration produces unfavorable contacts between the cyclopentyl ring and these active-site residues. In the case of the *N*-methyl-*N*-cyclohexylformamide and *N*-ethyl-*N*-cyclohexylformamide, the methyl and ethyl groups are not capable of providing these interactions. Binding of *N*-cyclopentyl-*N*-cyclobutylformamide might be improved further by adding a propyl or butyl group at carbon 3 of the cyclopentyl ring.

As compared to the other enzymes, the β_1 enzyme displays the highest affinity for *N*-benzylformamide. The modeling of the cis conformation into the active site provides favorable contacts between the phenyl ring and residues Val318 and Leu309 (Figure 1B). This conformation also provides a cation$-\pi$ interaction between the

amide N−H and Phe-93 of the enzyme. The β_1 enzyme also shows very strong binding of *N*-heptylformamide. Modeling studies show that the *N*-heptyl chain provides good contacts with Val-318 and Leu-309 of the enzyme.

Modeling suggests that the narrow active site in *Hs*ADH σ could have several hydrophobic contacts with the *N*-heptylformamide (Figure 1C). Comparison of the three-dimensional structures of the β and σ enzymes shows that the presence of Met-57, Met-141, and Phe-309 in the σ enzyme narrows the binding pocket as compared with the β enzyme, which has leucine residues at these positions.[10] The effect of these substitutions for binding of *N*-heptylformamide is not apparent, however, as the σ and β_1 enzymes have similar binding affinity. On the other hand, steric hindrance due to Met-57 and Met-141 in the σ enzyme could be responsible for the 10-fold or more decreases in affinity for the branched chain derivatives as compared to the linear alkyl chains. The 100-fold decrease in the affinity of the σ enzyme for *N*-1-methylheptylformamide as compared to that for *N*-heptylformamide is somewhat surprising as the σ enzyme has good catalytic activity with *all-trans*-retinol, and we would expect the *N*-1(*S*)-methylheptylformamide (trans conformation) could bind in a manner similar to *all-trans*-retinol.[13]

The γ_2 enzyme was most potently inhibited by *N*-1-methylheptylformamide, and the branched chain derivatives were better inhibitors than the linear alkyl compounds. The three-dimensional structure of this enzyme is not known, but the amino acid residues at the active site (Table 1) resemble those of the horse enzyme, for which structures of several complexes are available. The γ_2 and horse enzymes are the only ones in this group with Ser-48, rather than Thr-48, and there should be more space to accommodate the branched compounds. For the γ_2 enzyme, however, there appears to be even more space than in the horse enzyme, as the disubstituted derivatives were good inhibitors, albeit not as good as they are for the α enzyme. The γ enzyme is the only one of these enzymes with good activity with steroids (oxidizing 3β-hydroxyl groups), indicating a relatively large active-site pocket.[14]

The three-dimensional structure of the π enzyme is not known, but the inhibition studies provide some indication of the active-site topology. The π enzyme has relatively lower affinity for most of the formamides, and no compound had a K_i of less than 1 μM. The modeling with *N*-cyclohexylformamide does not indicate any steric conflicts between the cyclohexyl ring and the enzyme active site that would explain the lower affinity. The π enzyme shows good binding affinity for *N*-cyclohexylmethylformamide, indicating that the methylene extension can position the cyclohexyl ring to make favorable hydrophobic contacts with active-site residues. The relatively high affinity of the π enzyme for the disubstituted formamides provides evidence that the active site near the catalytic zinc is somewhat larger than predicted from the modeling studies.

These studies provide data for potential therapeutic agents and for correlating structure and function of the human alcohol dehydrogenases. The inhibition constants determined in this study are thermodynamic dissociation constants that reflect the energetics of the multiple binding interactions. In contrast, K_m values for substrates are steady-state kinetic constants. Thus, the active-site topology is better defined with inhibitors than with substrates. Although we can model the binding, observed ligand positions may be different from those expected from modeling because the differences in energetics among binding modes are small. X-ray crystallography of selected complexes would better define the interactions.

The inhibitors developed in this study have not been tested in animals, but other formamides and amides have been found to be effective in rats.[6,15,16] For broad spectrum inhibition of most alcohol dehydrogenases, *N*-cyclohexylmethylformamide and *N*-heptylformamide would appear to be the best inhibitors, but the *N*-cyclopentyl- and *N*-isopropylformamides might also have suitable pharmaceutical properties. After the substrate specificities of the alcohol dehydrogenases are further defined, selective inhibitors may be chosen to prevent oxidation of specific alcohols. For treatment of methanol poisoning, the β and γ enzymes should be inhibited, as these are abundant in the liver and have the highest catalytic activity.[17,18] Although we have found good inhibitors that exploit the specificities in the active sites, more potent inhibitors might be prepared.

Toxicity and metabolism of the new inhibitors have not been evaluated, but the LD_{50} for *N*-cyclohexylformamide in mice is 320 mg/kg,[19] which is 25 times higher than the dose expected to be required to produce 90% inhibition of the class I alcohol dehydrogenases. Since the formamides are uncompetitive inhibitors, the relative velocity of the enzymes, at saturating levels of alcohol is given by the relationship, $v = V/(1 + [I]/K_i)$.

Experimental Section

Materials. Crystalline horse liver alcohol dehydrogenase, NAD^+, and NADH were purchased from Boehringer Mannheim. Clones for the expression of *Hs*ADH α, β_1, and σ were obtained from Dr. Thomas D. Hurley (Indiana University School of Medicine), and the vectors for the γ_2 and π enzymes were from Dr. Jan-Olov Höög (Karolinska Institutet). *N*-Cyclohexylformamide and other reagents were obtained from Aldrich Chemical Co. *N*-Cyclohexylmethylformamide and *N*-isobutylformamide were prepared as described previously.[8] The primary and secondary amines were synthesized and isolated according to the procedure of Borch et al.[20] Compounds were characterized using a Varian 500 MHz NMR spectrometer. Multiple resonances are due to the cis−trans isomerism of the formamides. Elemental analyses were performed by Galbraith Laboratories.

Protein Purification. The human alcohol dehydrogenases were prepared essentially as described.[21,22] Protein homogeneity was established by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Enzyme activity was determined spectrophotometrically by following the change in absorbance at 340 nm due to NADH production ($\epsilon_{340} = 6.22$ mM^{-1} cm^{-1}). The specific activities of the purified enzymes in the standard assay with 2.4 mM NAD^+ and 33 mM ethanol in 0.1 M sodium glycine, pH 10, at 25 °C were comparable to the literature values.

Kinetic Studies. The inhibition constants were determined in 46 mM sodium phosphate and 0.25 mM EDTA, pH 7.0, at 25 °C by measuring the change in absorbance at 340 nm due to NADH. The kinetics of *Eq*ADH and *Hs*ADH α were also studied in 33 mM sodium phosphate and 0.25 mM EDTA, pH 8.0, at 25 °C, and it was found that the inhibition constants were about the same as at pH 7. The substrates used for assays differed with the various enzymes in order to obtain optimal activity. The coenzyme concentration was held constant at 0.2 mM NADH or 2.0 mM NAD^+. The concentrations

of inhibitor were varied over at least a 3-fold range, and 20−25 different assay conditions, with duplicates, were tested. The inhibition data were fitted to the equations for competitive or uncompetitive inhibition with the appropriate computer programs.[23]

Molecular Modeling. Molecular modeling was carried out using the computer program O.[24] Coordinates for the human enzymes were obtained from the Brookhaven Protein Database (entries: 1HDX, 1AGN). All inhibitors were built and minimized in SYBYL (Tripos Associates).

Synthesis of Formamides. The primary formamides were prepared by refluxing the amine with 88% HCOOH, removing excess HCOOH and H_2O under reduced pressure, and distilling the product. Attempts to synthesize the secondary formamides by the same procedure gave low yields and contaminating formate salts. Thus, an improved procedure was used. The amine was added dropwise to an excess of 88% formic acid. The reaction mixture was refluxed for 1.5 h, and excess formic acid was removed under reduced pressure. The formate salt was suspended in toluene and refluxed with stirring for 2 h before removing the toluene/H_2O azeotrope by distillation at 88 °C. Reflux and distillation were repeated until the ^{1}H NMR of the reaction mixture indicated that the reaction had gone to at least 60−70% completion. The excess toluene was removed under reduced pressure. The residue was dissolved in $CHCl_3$, and the organic layer was washed with water, dried with $MgSO_4$, and filtered. The solvent was removed under reduced pressure, and the product was applied to a silica gel column and eluted with ethyl acetate/hexane (60:40). The formamides were isolated as oils, with the exception of *N*-benzylformamide, which was a solid. The oils were not dried and contained fractional amounts of water. Final yields ranged from 30 to 40%.

***N*-Cyclopropylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.29 (d, 0.4H, CHO), 8.16 (s, 0.6H, CHO), 5.94−5.74 (s, broad, 0.4H, NH,), 6.17−5.96 (s, broad, 0.6H, NH), 2.81−2.67 (m, 0.6H, CH), 2.67−2.56 (m, 0.4H, CH), 0.89−0.73 (m, 2H, CH_2), 0.70−0.46 (m, 2H, CH_2). Anal. ($C_4H_7NO\cdot{}^1/_4H_2O$) C, H, N.

***N*-Cyclobutylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.09 (m, 1H, CHO), 6.25−6.01 (s, broad, 0.3H, NH), 6.00−5.62 (s, broad, 0.7H, NH), 4.55−4.40 (m, 0.7H, CH), 4.07−3.92 (m, 0.3H, CH), 2.41−2.26 (m, 2H, CH_2), 2.04−1.84 (m, 2H, CH_2), 1.80−1.61 (m, 2H, CH_2). Anal. Calcd for $C_5H_9NO\cdot{}^1/_6H_2O$: C, 58.97; H, 9.21; N, 13.75; Found: C, 59.47; H, 9.51; N, 13.01.

***N*-Cyclopentylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.17 (m, 1H, CHO), 6.13−5.71 (d, broad, 1H, NH), 4.34−4.18 (m, 0.8H, CH), 3.90−3.77 (m, 0.2H, CH), 2.06−1.89 (m, 2H, CH_2), 1.76−1.52 (m, 4H, CH_2), 1.52−1.31 (m, 2H, CH_2). Anal. ($C_6H_{11}NO\cdot{}^1/_{10}H_2O$) C, H, N.

***N*-Benzylformamide:**[7] ^{1}H NMR ($CDCl_3$) δ 8.28 (s, 0.9H, CHO), 8.20 (d, 0.1H, CHO), 7.40−7.22 (m, 5H), 6.05−5.79 (s, broad, 1H, NH), 4.49−4.46 (d, 1.8H, C*H*$_2$NH), 4.42−4.39 (d, 0.2H, C*H*$_2$NH).

***N*-Propylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.14 (s, 0.8H, CHO), 8.0−7.92 (d, 0.2H, CHO), 6.51−6.22 (s, broad, 1H, NH), 3.25−3.14 (m, 1.6H, C*H*$_2$NH), 3.14−3.06 (m, 0.4H, C*H*$_2$NH), 1.59−1.43 (m, 2H, $CH_3CH_2CH_2NH$), 0.94−0.81 (t, 3H, CH_3). Anal. Calcd for $C_4H_9NO\cdot 0.4H_2O$: C, 50.93; H, 10.47; N, 14.85. Found: C, 51.17; H, 10.39; N, 13.80.

***N*-Isopropylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.14−8.00 (m, 1H, CHO), 6.23−5.99 (s, broad, 0.2H, NH), 5.94−5.65 (s, broad, 0.8H, NH), 4.23−4.05 (m, 0.8H, $CH_3CH(NH)CH_3$), 3.76−3.59 (m, 0.2H, $CH_3CH(NH)CH_3$), 1.27−1.10 (m, 6H, $CH_3CH(NH)CH_3$). Anal. ($C_4H_9NO\cdot{}^1/_2H_2O$) C, H, N.

***N*-Butylformamide:** ^{1}H NMR ($CDCl_3$), δ 8.18 (s, 0.8H, CHO), 7.96 (d, 0.2H, CHO), 6.21 (s, broad, 0.2H, NH), 5.61 (s, broad, 0.8H, NH), 3.30−3.23 (m, 1.6H, C*H*$_2$NH), 3.21−3.10 (q, 0.4H, C*H*$_2$NH), 1.6−1.2 (m, 4H, CH_2), 0.80−0.99 (t, 3H, CH_3). Anal. Calcd for $C_5H_{11}NO\cdot{}^1/_2H_2O$: C, 54.52; H, 10.98; N, 12.72. Found: C, 52.73; H, 9.75; N, 11.94.

***N*-Heptylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.22 (s, 0.75H, CHO), 8.06−7.90 (d, 0.25H, CHO), 6.12−5.82 (d, broad, 1H, NH), 3.31−3.21 (m, 1.5H, C*H*$_2$NH), 3.2−3.13 (m, 0.5H, C*H*$_2$-NH), 1.58−1.44 (m, 2H, CH_2), 1.37−1.16 (m, 8H, CH_2), 0.93−0.77 (t, 3H, CH_3). Anal. ($C_8H_{17}NO\cdot{}^1/_4H_2O$) C, H, N.

***N*-1-Methylheptylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.17 (s, 0.7H, CHO), 8.08 (d, 0.3H, CHO), 5.79−5.57 (s, broad, 0.3H, NH), 5.52−5.32 (s, broad, 0.7NH), 4.16−3.99 (m, 0.7H, C*H*NH), 3.55−3.39 (m, 0.3H, C*H*NH), 1.57−1.38 (m, 2H), 1.36−1.09 (m, 11H), 0.97−0.81 (m, 3H). Anal. Calcd for $C_9H_{19}NO\cdot{}^1/_4H_2O$: C, 66.83; H, 12.15; N, 8.66. Found: C, 66.76; H, 11.48; N, 8.63.

***N*-Cyclopropyl-*N*-heptylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 0.8H, CHO), 8.09 (s, 0.2H, CHO), 3.33−3.24 (t, 1.6H, C*H*$_2$NH), 3.17−3.06 (t, 0.4H, C*H*$_2$NH), 2.70−2.58 (m, 0.8H, ring-CH), 2.55−2.45 (m, 0.2H, ring-CH), 1.66−1.51 (m, 2H, *n*-heptyl-CH_2), 1.39−1.19 (m, 8H, *n*-heptyl-CH_2), 0.92−0.81(t, 3H, CH_3), 0.82−0.73 (m, 2H, ring-CH_2), 0.72−0.59 (m, 2H, ring-CH_2). Anal. ($C_{11}H_{21}NO\cdot{}^1/_{10}H_2O$) C, H, N.

***N*,*N*-Dicyclobutylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.3 (s, 1H, CHO), 4.5−4.4 (m, 1H, CH), 4.0−3.8 (m, 1H, CH), 2.4−2.1 (m, 8H, CH_2), 1.8−1.57 (m, 4H, CH_2). Anal. ($C_9H_{15}NO\cdot{}^1/_6H_2O$) C, H, N.

***N*-Cyclopentyl-*N*-cyclopropylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.29 (s, 0.8H, CHO), 8.22 (s, 0.2H, CHO), 4.47−4.32 (m, 1H), 2.62−2.46 (m, 1H), 1.90−1.79 (m, 2H), 1.78−1.50 (m, 6H), 0.85−0.74 (m, 2H), 0.74−0.62 (m, 2H). Anal. ($C_4H_{15}NO\cdot{}^1/_6H_2O$) C, H, N.

***N*-Cyclopentyl-*N*-cyclobutylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.31 (s, 0.6H, CHO), 8.2 (s, 0.4H, CHO), 4.42−4.26 (m, 1H), 3.93−3.75 (m, 1H), 2.41−2.07 (m, 4H), 1.87−1.45 (m, 10H). Anal. ($C_{10}H_{17}NO\cdot{}^1/_6H_2O$) C, H, N.

***N*,*N*-Dicyclopentylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.23 (s, 1H, CHO), 4.16−4.00 (m, 1H, CH), 3.76−3.63 (m, 1H, CH), 2.01−1.86 (m, 2H, CH_2), 1.86−1.67 (m, 8H, CH_2), 1.64−1.46 (m, 6H, CH_2). Anal. ($C_{11}H_{19}NO\cdot{}^1/_8H_2O$) C, H, N.

***N*-Cyclohexyl-*N*-methylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.15 (s, 0.7H, CHO), 7.90 (s, 0.3H, CHO), 4.30−4.15 (m, 0.7H, CH), 3.37−3.19 (m, 0.3H, CH), 2.89 (s, 0.9H, CH_3), 2.80 (s, 2.1H, CH_3), 1.91−1.78 (m, 2H, CH_2), 1.76−1.63 (m, 4H, CH_2), 1.61−1.27 (m, 4H, CH_2). Anal. ($C_8H_{15}NO\cdot{}^1/_3H_2O$) C, H, N.

***N*-Cyclohexyl-*N*-ethylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.16 (s, 0.7H, CHO), 8.07 (s, 0.3H, CHO), 4.10−3.94 (m, 0.3H, ring-CH), 3.33−3.22 (m, 2H, CH_3CH_2N), 3.22−3.13 (m, 0.7H, ring-CH), 1.88−1.59 (m, 6H, ring-CH_2), 1.55−1.41 (m, 2H, ring-CH_2), 1.39−1.22 (m, 2H, ring-CH_2), 1.22−1.16 (t, 0.9H, CH_3), 1.15−1.08 (t, 2.1H, CH_3). Anal. ($C_{19}H_{17}NO\cdot{}^1/_3H_2O$) C, H, N.

***N*-Cyclohexyl-*N*-propylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.21 (s, 0.7H, CHO), 8.1 (s, 0.3H, CHO), 4.0−3.9 (m, 0.3H, ring-CH), 3.22−3.18 (t, 2H, CH_2N), 3.09−3.04 (m, 0.7H, ring-CH), 1.89−1.39 (m, 8H, ring-CH_2), 1.39−1.22 (m, 2H, ring-CH_2), 1.19−1.03 (m, 2H, $CH_3CH_2CH_2N$), 0.96−0.81 (m, 3H, CH_3). Anal. ($C_{10}H_{19}NO\cdot{}^1/_3H_2O$) C, H, N.

***N*-Cyclohexyl-*N*-isopropylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.26 (s, 0.4H, CHO), 8.14 (s, 0.6H, CHO), 4.39−4.22 (m, 0.6H, ring-CH), 3.91−3.76 (m, 0.4H, ring-CH), 3.66−3.55 (m, 0.4H, $CH_3CH(N)CH_3$), 3.13−2.99 (m, 0.6H, $CH_3CH(N)CH_3$), 1.90−1.41 (m, 10H, ring-CH_2), 1.38−1.23 (d, 3H, CH_3), 1.22−1.16 (d, 3H, CH_3). Anal. ($C_{10}H_{19}NO\cdot{}^1/_8H_2O$) C, H, N.

***N*-Cyclopentyl-*N*-propylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.22 (s, 0.7H, CHO), 8.12 (s, 0.3H, CHO), 4.40−4.22 (m, 0.3H, ring-CH), 3.87−3.71 (m, 0.7H, ring-CH), 3.23−3.08 (m, 2H, CH_2), 1.96−1.84 (m, 2H, CH_2), 1.82−1.70 (m, 2H, CH_2), 1.68−1.53 (m, 6H, CH_2), 0.96−0.87 (m, 3H, CH_3). Anal. ($C_9H_{17}NO\cdot{}^1/_4H_2O$) C, H, N.

***N*-Cyclohexyl-*N*-cyclopentylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.29 (s, 0.5H, CHO), 8.19−8.13 (s, 0.5H, CHO), 4.29−4.11 (m, 0.5H, CH), 3.91−3.75 (m, 0.5H, CH), 3.73−3.58 (m, 0.5H, CH), 3.11−2.93 (m, 0.5H, CH), 2.00−1.41 (m, 16H, CH_2), 1.39−1.22 (m, 2H, CH_2). Anal. ($C_{12}H_{21}NO\cdot{}^1/_8H_2O$) C, H, N.

***N*-Cyclohexyl-*N*-cyclopropylformamide:** ^{1}H NMR ($CDCl_3$) δ 8.29 (s, 0.9H, CHO), 8.22 (s, 0.1H, CHO), 4.16−4.02 (m, 0.9H, CH), 3.26−3.07 (m, 0.1, CH), 2.56−2.48 (m, 0.9H, CH), 2.48−2.43 (m, 0.1H, CH), 1.93−1.77 (m, 2H, CH_2), 1.76−1.59 (m, 6H, CH_2), 1.46−1.28 (m, 2H, CH_2), 0.86−0.76 (m, 2H, CH_2), 0.75−0.63 (m, 2H, CH_2). Anal. ($C_{10}H_{17}NO\cdot{}^1/_8H_2O$) C, H, N.

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(1) Abbreviations: *Hs*ADH, human (*Homo sapiens*) alcohol dehydrogenase; *Eq*ADH, horse (*Equus caballus*) alcohol dehydrogenase.
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