Design of Inhibitors from the Three-Dimensional Structure of Alcohol Dehydrogenase. Chemical Synthesis and Enzymatic Properties†

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Abstract: Inhibitors of liver alcohol dehydrogenase were designed from the three-dimensional structure of the enzyme. The ligand to the catalytic zinc ion is an amide group or, better, a formamide group. With the latter function, a hydrogen bond between the NH group and the hydroxyl group of Ser-48 may be formed. The hydrophobic substrate binding site brings structural restraints. α-ω bifunctional molecules show good inhibitory properties possibly due to the interactions with polar residues at the entrance of the substrate binding site.

Introduction

The design of a molecule which should bind to a receptor has for a long time been based on structural analogy with the natural effector or has relied on the structure–activity relationship.

From the time three-dimensional structures became available, more rational methods appeared.[1] Using wire models, a molecular modeling approach was successfully applied to the binding site of human hemoglobin,[2] and potential antisickling agents based on the structure of this protein have been described.[3] Recently, molecular surface models were used to predict the binding affinities of thyroid hormone analogues to prealbumin,[4] while a computational method was published for a geometric approach to macromolecule–ligand interactions.[5a] The three-dimensional molecular models of *E. coli* dihydrofolate reductase–methotrexate complex was used to design analogues of trimethoprim that had higher affinity for dihydrofolate reductase than that of trimethoprim. The modification was to introduce in the molecule of trimethoprim an acid group which interacts with a cationic group as does methotrexate.[5b]

We present here the results on the design of potent inhibitors, from the three-dimensional structure of a given receptor. Alcohol dehydrogenase appeared as a good candidate with respect to biochemical and molecular knowledge and to pharmacological and toxicological interests.

Specific inhibition of alcohol dehydrogenase might give a better insight of the physiological role of the enzyme and will prevent poisoning due to methanol, ethylene glycol, or an excess of ethanol ingestion.[6]

We initially made two choices. The first was to prepared reversible inhibitors, because irreversible inhibitors, even if they are affinity labels to alcohol dehydrogenase, may react with other reactive groups as second-order reagents and thus be nonselective. The second was to prepare inhibitors binding to the substrate binding site and not to the coenzyme binding site. NAD^+ and NADH are coenzymes for a large number of enzymes, and X-ray structure determinations of dehydrogenases have shown the similarity of the coenzyme binding sites.[7] The search for suicide inhibitors of $NAD(P)^+$-dependent dehydrogenase seems to have been unsuccessfull until now.

From the alcohol dehydrogenase–NADH–dimethyl sulfoxide triclinic complex, the structure of the holoenzyme has been solved and refined at 2.9-Å resolution.[8] In addition, several other complexes of the enzyme with coenzyme or coenzyme analogues and substrates or inhibitors have been studied to high resolution.[9,10] These investigations have provided an accurate description of the interactions between the protein and its ligands. In all triclinic complexes, in which the enzyme undergoes conformational change, the coenzyme or coenzyme analogue is identically bound to the protein,[11] while the other ligands are located in the substrate binding site. All substrates or inhibitors are bound to the catalytic zinc atom through an electron-donating atom (oxygen or nitrogen) at distances ranging from 2.0 to 2.2 Å.

From structural information, the substrate specificity of horse liver alcohol dehydrogenase has been rationalized in the form of a diamond lattice.[12] An irreversible inhibitor has been designed in order to bind to the substrate binding site and to react with thioether of Met-306. Inactivation of the enzyme by this reagent has been reported.[13]

Pyrazole and 4-substituted pyrazole are powerful inhibitors of alcohol dehydrogenase in the presence of oxidized coenzyme.[14a] Crystallographic studies with NAD^+, pyrazole, or 4-iodopyrazole[15] have shown that the large stability of the LADH–NAD^+–pyrazole complex arises from the fact that the pyrazole molecule is bound through one nitrogen atom to the catalytic zinc atom and through its second nitrogen to the C-4 atom of the pyridinium ring. Pyrazole is toxic in vivo,[16] but 4-substituted pyrazoles seem to be devoid of toxicity. Aldoximes have been shown recently to act

† Dedicated to Professor E. Lederer for his 75th birthday.

(1) Gund, P.; Andose, J. D.; Rhodes, J. B.; Smith, G. M. *Science* **1980**, *208*, 1425–1431. Langridge, R.; Ferrin, T. E.; Kuntz, I. D.; Connolly, M. L. *Ibid.* **1981**, *211*, 661–666.
(2) Beddell, C. R.; Goodford, P. J.; Norrington, F. E.; Wilkinson, S.; Wootton, R. *Br. J. Pharmacol.* **1976**, *57*, 201–209.
(3) Klotz, I. M.; Haney, D. N.; King, L. C. *Science* **1981**, *213*, 724–731.
(4) Blaney, J. M.; Jorgensen, E. C.; Connolly, M. L.; Ferrin, T. E.; Langridge, R.; Oatley, S. J.; Burridge, J. M.; Blake, C. C. F. *J. Med. Chem.* **1982**, *25*, 785–790.
(5) (a) Kuntz, I. D.; Blaney, J. M.; Oatley, S. J.; Langridge, R.; Ferrin, T. E. *J. Mol. Biol.* **1982**, *161*, 269–288. (b) Kuyper, L. F.; Roth, B.; Baccanari, D. P.; Ferone, R.; Beddell, C. R.; Champness, J. N.; Stammers, D. K.; Dann, J. G.; Norrington, F. E. A.; Baker, D. J.; Goodford, P. J. *J. Med. Chem.* **1982**, *25*, 1120–1122.
(6) Moeschlin, S. "Klinik und Therapie der Vergiftungen"; Georg Thieme Verlag: Stuttgart, 1972; pp 205–215, 222–223.
(7) Ohlsson, I.; Nordström, B.; Bränden, C. I. *J. Mol. Biol.* **1974**, *89*, 339–354.
(8) Eklund, H.; Samama, J. P.; Wallén, L.; Bränden, C. I.; Åkeson, Å.; Jones, T. A. *J. Mol. Biol.* **1981**, *146*, 561–587.
(9) Cedergren-Zeppezauer, E.; Samama, J. P.; Eklund, H. *Biochemistry* **1982**, *21*, 4895–4908.
(10) Eklund, H.; Plapp, B. V.; Samama, J. P.; Bränden, C. I. *J. Biol. Chem.* **1982**, *257*, 14349–14358.
(11) Eklund, H.; Samama, J. P.; Jones, T. A. *Biochemistry*, accepted for publication.
(12) Dutler, H.; Bränden, C. I. *Bioorg. Chem.* **1981**, *10*, 1–13.
(13) Chen, W. S.; Plapp, B. V. *Biochemistry* **1978**, *17*, 4916–4922.
(14) (a) Theorell, H.; Yonentani, T.; Sjöberg, B. *Acta Chem. Scand.* **1969**, *23*, 255–260. Tolf, B. R.; Dahlbron, R.; Theorell, H.; Åkeson, Å. *Acta Chem. Scand., Ser. B* **1982**, *36*, 101–107. (b) Sigman, D. S.; Frolich, M.; Anderson, R. E. *Eur. J. Biochem.* **1982**, *126*, 523–529.
(15) Eklund, H.; Samama, J. P.; Wallén, L. *Biochemistry* **1982**, *21*, 4858–4866.
(16) Horvath, E.; Kovacs, J. *Science* **1977**, *199*, 1209–1210.

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as inhibitors binding to the LADH–NAD^+ complex.[14b]

The ordered bi–bi mechanism for alcohol dehydrogenase implies that the enzyme–coenzyme complex is formed at first in either direction of the reaction sequence. Thus, acetaldehyde competitive inhibitors, binding to the LADH–NADH complex, are kinetically noncompetitive inhibitors toward ethanol. In the oxidation process of ethanol, they would reduce the overall rate of the reaction and thus be more potent at high ethanol concentration.[17]

In the pioneering work on alcohol dehydrogenase, it was discovered that amides inhibited the catalytic reaction by forming enzyme–NADH–amide complexes.[18] We report in this paper the design from structural information of amides and formamides specifically suited to the substrate binding site of alcohol dehydrogenase.

Experimental Section

1. Biochemical Aspects. The alcohol dehydrogenase (E.C.1.1.1.1) from horse liver was purchased from Boehringer-Mannheim as a crystalline suspension in 20 mM potassium phosphate buffer pH 7 containing 10% ethanol. After centrifugation, the crystals were dissolved in 0.1 M potassium phosphate buffer pH 7.5. After two dialyses against the same buffer, some undissolved material was removed by centrifugation. The active site content was routinely determined.[19]

NAD^+ and NADH were products of Boehringer-Mannheim. Tetramethylurea from Aldrich was twice vacuum distilled. Some lots contained an unidentified strong inhibitor of the enzyme and were disregarded. Acetaldehyde was prepared from paraldehyde (Fluka) by depolarization with traces of sulfuric acid and distillation. Purified acetaldehyde was obtained after three successive distillations. Ethanol of the purest grade available (Prolabo) was used.

The infrared spectra were recorded by using a Perkin-Elmer 177 spectrometer. The ^1H NMR spectra were recorded on a Perkin-Elmer R12 (60 MHz) or R32 (90 MHz) spectrometer. Chemical shifts are expressed in parts per million (δ) and the coupling constants in hertz. The internal reference was tetramethylsilane. A Cary 118 spectrophotometer was used for recording the UV spectra. The carbon and hydrogen determinations were done by the microanalytical division of the C.N.R.S. in Strasbourg (Mme. Francois). For all compounds, the values found for C, H, and N were within 0.1% of theoretical values.

Enzymic Test. The enzymic tests were performed in a 0.1 M potassium phosphate buffer pH 7.5 by following the absorption variation at 340 nm. The initial concentrations of NAD^+ and NADH were 5.6 and 0.2 mM, respectively. The concentrations of ethanol or acetaldehyde were varied in the range 5.7–1.2 and 0.5–0.12 mM, respectively. Tetramethylurea was used when the tested inhibitor did not dissolve in the test medium. A solution of the compound in tetramethylurea was prepared and added to the test medium. At a concentration of 10 μL for 1 mL of test medium, the tetamethylurea did not inhibit the enzyme. The inhibition constants were determined according to Lineweaver–Burk.[20]

2. Chemical Aspects. Preparation. Standard procedures (labeled from A to U) were used for the different chemical steps and will be reported only once. The preparation of each compound will therefore refer to a given procedure. Under standard extraction, we mean washing of the organic layer to neutrality, then once with brine, drying over sodium sulfate, and removal of the solvent under reduced pressure.

We indicate in the tables the reactions used in the preparation and the data of the final compounds. The standard procedures (A–U) used for the preparation follow.

(A) Phenylacetamide. A suspension of phenylacetonitrile (5 g, 42.7 mmol) in 2 N chlorhydric acid (10 mL) was maintained at 40 °C for 1 h, at 15 °C for 0.5 h, and at 4 °C for 0.5 h. The crystalline precipitate was filtered, washed with water, and dried. By recrystallization in toluene–water, phenylacetamide was obtained (4.3 g, 31.8 mmol, yield 75%).

(B) *p*-Pentoxyphenylacetamide. A solution of *p*-phentoxyphenylacetonitrile (500 mg, 2.5 mmol), prepared according to procedure *T*, in acetone (8 mL) was added to a 10% aqueous solution of sodium carbonate (0.2 mL) and to 15% hydrogen peroxide solution (1.5 mL). After the mixture was stirred for 3 days at 20 °C, acetone was removed and the crystalline product was filtered. The amide was recrystallized in methanol–water, yield 67%.

(C) 3-Phenylpropionamide. A mixture of 3-phenylpropionic acid (3 g, 20 mmol) and thionyl chloride (6 mL, 83 mmol) was refluxed for 2 h. After removal of the excess of thionyl chloride, the acid chloride was dissolved in toluene (5 mL) and the solution was saturated with ammonia. After 1 h, the amide was filtered and recrystallized three times from water.

(D) *p*-Butoxyphenylacetamide. A mixture of urea (1 g, 16.6 mmol) and *p*-butoxyphenylacetic acid (0.33 g, 1.6 mmol), prepared according to procedure *I*, was heated to 200 °C for 3 h. After removal of urea with water, the amide was recyrstallized in toluene, yield 70%.

(E) *p*-Butoxyphenylacetamide. Methyl *p*-butoxyphenylacetate (1 g, 4.5 mmol) was dissolved in an ammonia-saturated solution of sodium methylate (prepared from 50 mg of sodium and methanol (10 mL)). Ammonia was passed through the solution for 2 h. After 24 h, water was added and the amide was filtered and recrystallized in methanol–water, yield 60%.

(F) *N*-Benzylformamide. A solution of benzylamine (5 g, 46.7 mmol) in formic acid (10 mL) was heated for 8 h at 100 °C. Excess of reagent was evaporated under vacuum until the product crystallized. The formamide was recrystallized in methanol–water, yield 60%.

(G) *N*-Benzylacetamide. A solution of benzylamine (3 g, 28 mmol) and acetic anhydride (9 mL) was heated for 10 h at 100 °C. Excess of reagent was evaporated under vacuum until the product crystallized. The *N*-benzylacetamide was recrystallized in methanol–water, yield 70%.

(H) *N*-Benzyltrifluoroacetamide. A solution of benzylamine (3 g, 28 mmol) in trifluoroacetic anhydride (4 mL) and tetrahydrofuran (5 mL) was refluxed for 12 h. Solvent and excess of reagent were removed by vacuum distillation. The product was recrystallized in methanol–water, yield 70%.

(I) *p*-Butoxyphenylacetamide. A suspension of *p*-hydroxyacetonitrile (4 g, 30 mmol) and anhydrous potassium carbonate (6.2 g, 45 mmol) in butyl bromide (8 g, 58.3 mmol) and acetone (8 mL) was treated with stirring under reflux for 8 h. After evaporation, the residue was extracted with ether. The *p*-butoxyphenylacetonitrile used as such was converted to the amide according to procedure B. The overall yield was 51%.

(J) *N,N*-Dimethyl-*p*-butoxyphenylacetamide. A suspension of *p*-hydroxyphenylacetic acid (3 g, 22 mmol), potassium hydroxide (3 g, 53.5 mmol), and *n*-butyl bromide (5 g, 36.5 mmol) in methanol (10 mL) was refluxed for 8 h. Then a 13 N solution of potassium hydroxide in water (2 mL) was added in order to saponify the ester. The reflux was continued for 1.5 h. After the reaction medium was cooled, dilute hydrochloric acid was added until pH was acidic. The precipitated acid was filtered and dried. Its solution in thionyl chloride (10 mL) was refluxed for 1 h. Excess reagent was removed under vacuum. The acid chloride was dissolved in toluene (60 mL), and dimethylamine (5 mL) was added. The amide was obtained by distillation. The overall yield was 21%.

(K) *m*-Butoxyphenylacetamide. A solution of *m*-hydroxybenzylic alcohol (2.5 g, 20 mmol), butyl iodide (3.7 g, 20 mmol), and potassium carbonate (2.9 g, 21 mmol) in acetone (12 mL) was refluxed for 15 h. After evaporation, the residue was extracted with ether and dried over sodium sulfate; the ether was removed. The alcohol (3 g) was treated with thionyl chloride (6 mL). After a reflux of 2 h, excess reagent was removed by distillation. The obtained *m*-butoxybenzyl chloride was dissolved in acetone (8 mL), and a solution of potassium cyanide (2 g, 30.7 mmol) and potassium iodide (0.5 g) in water (10 mL) was added. After a reflux of 36 h, the reaction was complete according to thin layer chromatography. The solvent was removed and the residue extracted with ether. After evaporation of ether, chromatography on silica gel (30 g, eluent hexane–ether) was performed. The *m*-butoxyphenylacetonitrile was obtained and converted to the amide using procedure **B**. The amide was recrystallized in methanol–water overall yield 18%.

(L) *p*-(7-Carbamoylheptamethylenoxy)phenylacetamide. Tosyl chloride (1 g, 5.2 mmol) was added at 0 °C to a solution of methyl 8-hydroxyoctanoate (0.9 g, 5.2 mmol) in pyridine (2 mL). After 48 h at 4 °C, the pyridine was removed under vacuum and the residue was extracted with ether. The ethereal layer was washed five times with water and dried over sodium sulfate, upon solvent removal, the tosylate (1 g) was obtained and used without further purification. A suspension of the crude tosylate (0.34 g), *p*-hydroxyphenylacetonitrile (0.13 g, 1 mmol), and potassium carbonate (0.2 g, 1.45 mmol) in acetone (1.5 mL) was refluxed for 8 h. The acetone was removed under vacuum and the residue extracted with ether. The obtained nitrile was converted to the amide according to procedure B. The methyl ester amide was recrystallized in methanol–H_2O, overall yield 28%. The methyl ester amide (50 g) dissolved in methanol (1 mL) was hydrolyzed with 1 N sodium hydroxide solution. After a reflux of 4 h, the solution was acidified with dilute hydrochloric acid. The acid–amide obtained as crystals was filtered, dried, and converted to the diamide using procedure D. The overall

(17) Christopherson, R. I.; Duggleby, R. G. *Eur. J. Biochem.* **1983**, *134*, 331–335.

(18) Winer, A. D.; Theorell, H. *Acta Chem. Scand.* **1960**, *14*, 1729–1742. Amides form complexes with alcohol dehydrogenase–NAD^+, but with lower affinity: Sigman, D. S.; Winer, A. D. *Biochim. Biophys. Acta* **1970**, *206*, 183–186.

(19) Theorell, H.; Yonetani, T. *Biochem. Z.* **1963**, *338*, 637–653.

(20) Lineweaver, H.; Burk, D. *J. Am. Chem. Soc.* **1934**, *56*, 658–666.

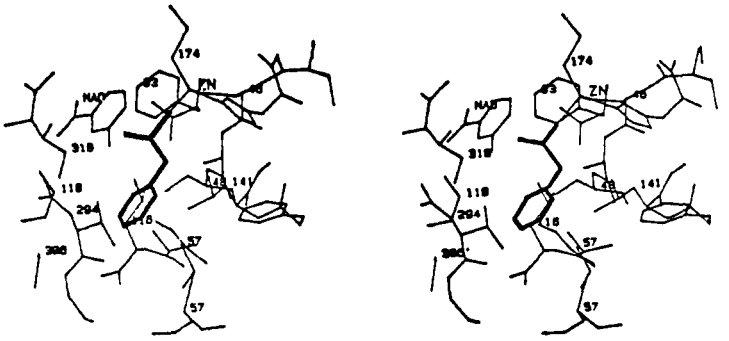
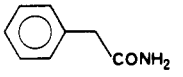
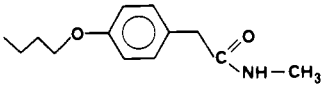
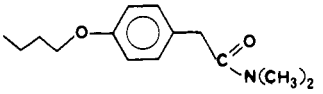
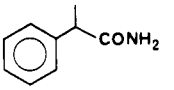
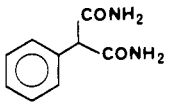
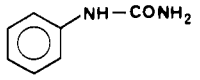


Figure 1. Stereodiagram of the bound phenylacetamide to liver alcohol dehydrogenase–NADH complex. The inhibitor structure is drawn in darker lines. The oxygen atom of the amide of the phenylacetamide acts as fourth ligand to zinc ion (ZN) and the nitrogen of the amide is oriented toward the nitrogen of the amide of NADH.

Table I. Inhibition. Derivatives of Phenylacetamide: Effects of Substitution Next to the Amide

compd	chemical structure	K_I (μ M)	mp (lit., °C)	prepn method	structure assignment
1		140	159–160 (159–160[a])	A	
2		200	95–96	J	*b*
3		1200	90[c]	J	*b*
4		900	92.5–93	C	
5		400	235–236 (233[d])	E	
6		670	146–148 (144[e])	*f*	

[a] Danis, C. H.; Carmack, M. *J. Org. Chem.* 1947, *12*, 76–78. [b] NMR data are given in the Experimental Section. [c] Boiling point under 1 mmHg. [d] Eijkman, J. F. *Chem. Zentr.* 1908, *II*, 1100–1101. [e] Davis, T. L.; Blanchard, K. C. *J. Am. Chem. Soc.* 1923, *45*, 1816–1820. [f] Commercially available.

yield starting from methyl 8-hydroxyoctanoate was 3%.

(M) 3-(*p*-Butoxyphenyl)propionohydrazide. The 3-(*p*-butoxyphenyl)propionic acid prepared in the procedure J was converted to its methyl ester. A solution of the acid (1.8 g, 8.1 mmol) in methanol (10 mL) saturated in hydrochloric acid was refluxed for 1 h. After removal of the solvent, the methyl ester was obtained and treated with hydrazine hydrate, 98% (0.35 g). After 2 h at 100 °C, the solid was filtered and washed with water. The hydrazide was recrystallized in methanol–water, overall yield 68%.

(N) 3-(*p*-Butoxyphenyl)thiopropionamide. A solution of *p*-hydroxybenzaldehyde (7 g, 57.4 mmol) potassium hydroxide (3.5 g, 62.5 mmol), and butyl bromide (13 g, 95 mmol) in methanol (25 mL) was refluxed for 24 h. Part of the reaction medium was removed under vacuum. Water was added and the standard extraction procedure was performed. *p*-Butoxybenzaldehyde was obtained.

The ylide was prepared from triphenylphosphine and chloroacetonitrile. A solution of triphenylphosphine (5 g, 19 mmol) and chloroacetonitrile (4.1 g) in toluene (15 mL) was refluxed for 12 h. After filtration, the precipitate was washed with toluene and dried. The cyanomethyltriphenylphosphonium chloride (5.9 g, 17.5 mmol) was obtained. A solution of this salt (5 g) in water (100 mL) was treated with 1 N sodium hydroxide solution (25 mL). The ylide (3.9 g) was filtered, dried, and used as such. A solution of the *p*-butoxybenzaldehyde (2.4 g) and the ylide (3.9 g) in toluene (30 mL) was refluxed for 4 h. After removal of the solvent, the residue was extracted with ether. The ethereal layer containing the nitrile was evaporated. Chromatography on silica gel (200 g, eluent CH_2Cl_2) was performed. The *p*-butoxycinnamonitrile was obtained (1.8 g, 3 mmol). The overall yield from *p*-hydroxybenzaldehyde was 58%.

p-Butoxycinnamonitrile (1.8 g) dissolved in ethyl acetate (15 mL) was reduced with hydrogen in the presence of 5% palladium on charcoal (50 mg). After consumption of 200 mL of hydrogen at 20 °C, the catalyst was removed by filtration and ethyl acetate was evaporated. The 3-(*p*-butoxyphenyl)propionitrile was distilled at 145 °C (15 mmHg).

(O) 11-Formylaminoundecanamide. A solution of 11-aminoundecanoic acid (1 g, 5 mmol) in a mixture of formic acid (2 mL) and acetic anhydride (1 mL) was treated at 90 °C for 12 h. Excess reagent was removed under vacuum and the residue recrystallized from acetone. To a suspension of the obtained acid (1 g, 4.36 mmol) in anhydrous THF (20 mL), triethylamine (0.50 g, 5 mmol) and ethyl chloroformate (0.54 g, 5 mmol) were added. After 12 h of stirring at 20 °C, the suspension was filtered and the filtrate added to an ammonia-saturated solution in THF (40 mL). After 0.5 h, the formamide–amide was filtered and recrystallized from acetone.

(P) 11-Guanidinoundecanamide Hemisulfate. The methyl ester was prepared from 11-aminoundecanoic acid using procedure M. A solution of amino ester (0.9 g, 4.2 mmol) and *S*-methylisothiouronium sulfate (1.3 g, 4.7 mmol) in methanol (20 mL) was refluxed for 5 h. After cooling to 20 °C, the precipitate was filtered and washed with water. The hemisulfate of methyl 11-guanidinoundecanoate (1.1 g) was obtained and

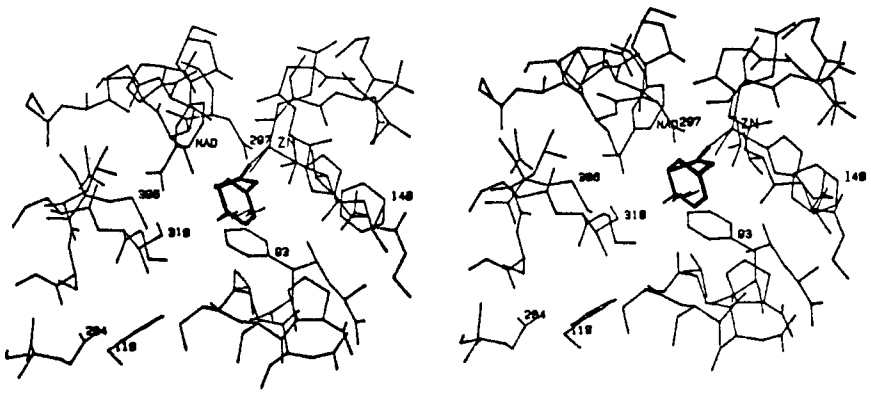
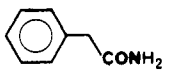
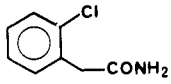
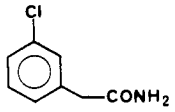
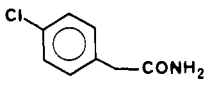
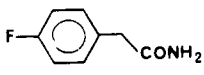
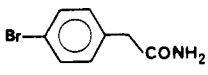
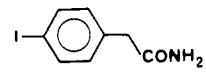


Figure 2. Stereodiagram of the bound *p*-butoxyphenylacetamide to liver alcohol dehydrogenase–NADH complex. The inhibitor structure drawn is in darker lines. The oxygen atom of the inhibitor acts as fourth ligand to zinc ion (ZN) and the nitrogen of the amide is oriented toward the nitrogen of the amide of NADH. (obtained as in Figure 1).

Table II. Inhibition. Substitutions on the Phenyl Ring of Phenylacetamide

compd	chemical structure	K_I (μM)	mp (lit.), °C	prepn method
1		140	159–160 (159–160[a])	A
7		140	177–178 (175[b])	C
8		90	130–131	C
9		20	175–175.5 (175[c])	C
10		64	156–157 (163–164[d])	C
11		20	192–193 (192–194[e])	A
12		8	209–210 (191[f])	C

[a] Danis, C. H.; Carmack, M. *J. Org. Chem.* 1947, *12*, 76–78. [b] Mehner, H. *J. Prakt. Chem.* 1900, *62*, 554–566. [c] Beilstein, F.; Kuhlberg, A. *Justus Liebigs Ann. Chem.* 1868, *147*, 349–350. [d] Olah, G.; Pavlath, A.; Kuhn, I. *Acta Chim. Acad. Sci. Hung.* 1955, *7*, 8592–8593. [e] Pschorr, R. *Chem. Ber.* 1906, *39*, 3117–3118. [f] Baskakov, Y. A.; Mel'Nikov, *Chem. Abstr.* 1954, *48*, 4477c.

converted to the amide by procedure E. The guanidino amide (0.5 g) was recrystallized in tetramethylurea. The overall yield from the methyl ester was 40%.

(Q) 11-Phthalimidoundecanamide. A mixture of 11-aminoundecanoic acid (2 g, 10 mmol) and phthalic anhydride (1.5 g, 10 mmol) was heated at 150 °C for 30 min. After cooling, thionyl chloride (3 mL) was added and the solution was refluxed for 1 h. Excess reagent was removed under vacuum. The residue was dissolved in toluene saturated with ammonia (50 mL). After 1 h, the precipitate was filtered and washed with water and toluene. The 11-phthalimidoundecanamide was recrystallized from acetic acid, overall yield 60%.

(R) *N*-(*p*-Butoxybenzyl)formamide. The *p*-butoxybenzonitrile was prepared using proceudre I. A solution of this nitrile (5.3 g, 27.2 mmol) in anhydrous ether (30 mL) was added dropwise at 4 °C to a solution of lithium aluminum hydride (1.2 g, 31.5 mmol) in anhydrous ether (50 mL). After 2 h, excess hydride was destroyed by cautious addition of small volumes of water. After filtration, the usual extraction procedure was performed. Starting with the crude amine, the *N*-(*p*-butoxybenzyl)formamide was prepared using procedure F, yield 33%. The overall yield from *p*-butoxybenzonitrile was 24%.

(S) *N*-Formyl-*N'*-propylglycinamide. A solution of glycine (5 g, 66.7 mmol) in formic acid (7.5 mL) was heated at 90 °C for 8 h. Excess reagent was removed under vacuum and the residue was sublimed. Crude *N*-formylglycine (2.3 g) was obtained. To a solution of the crude *N*-formylglycine (0.50 g) in tetrahydrofuran (5 mL) and DMF (5 mL), triethylamine (0.59 g) and ethyl chloroformate (0.63 g) were added. After 10 min, the suspension was filtered and propylamine (2 ml) was added to the filtrate. After 1 h, the solvent was removed under vacuum. Chromatography on silica gel (25 g; eluent: CH_2Cl_2/MeOH 9:1) was performed. *N*-Formyl-*N'*-propylglycinamide (0.22 g) was purified by sublimation, overall yield from glycine 10%.

(T) *N*-(*p*-Hexylbenzyl)formamide. A solution of 1-bromopentane (10 g, 67 mmol) and triphenylphosphine (5 g, 19 mmol) in tetrahydrofuran (8 mL) was heated at 90 °C for 12 h. After cooling the *n*-pentyltriphenylphosphonium bromide (6 g, 14.6 mmol) was filtered, yield 77%.

To a suspension of the phosphonium salt (4 g, 9.73 mmol) in anhydrous ether (30 mL) was added under argon a 2 M solution of butyllithium (5 mL). The salt dissolved slowly. After 2 h, 4-cyanobenzaldehyde (1.3 g, 10 mmol) was added. After 1 h, water was added and the standard extraction performed. The solvent from the ethereal layer was distilled and the residue extracted with hexane. The obtained 1-(*p*-cyanophenyl)hexene-1 in solution in ethyl acetate (10 mL) was reduced with hydrogen in the presence of 5% palladium on charcoal. After the consumption of hydrogen (140 mL at 20 °C) the catalyst was filtered and the solvent evaporated. The 1-(*p*-cyanophenyl)hexane was used as such in procedure R to prepare the corresponding amine which was converted to *N*-(*p*-hexylbenzyl)formamide using procedure F. The overall yield from 4-cyanobenzaldehyde was 10%.

(U) *p*-Arbutinylacetamide. A solution of *p*-arbutin (0.5 g, 1.8 mmol from Roth, Karlsruhe, RFA) and iodoacetamide (0.5 g, 2.7 mmol) in sodium methylate solution (prepared from 60 mg of sodium and 6 mL of methanol) was refluxed for 24 h. At 20 °C the suspension was filtered. The precipitate was washed with a small amount of methanol and acetone. The product was recrystallized from methanol-H_2O. The yield from *p*-arbutin was 55%.

NMR Assignments Follow.

2 (90 MHz, $CDCl_3$): δ 0.98 (t, 3 H), 1.2–1.9 (4 H), 2.75 (s, 2 H), 3.5 (s, 2 H), 3.94 (t, 2 H), 7.0 (AB system, J_{AB} = 9 Hz, $\Delta\delta$ 0.32 ppm, 4 H).

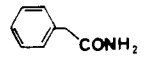
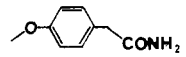
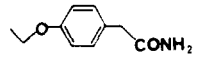
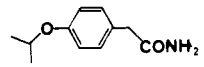
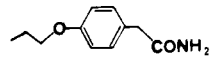
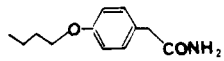
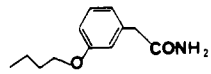
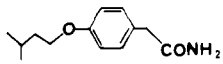
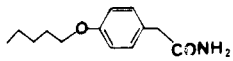
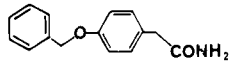
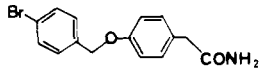
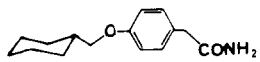
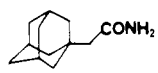
3 (60 MHz, $CDCl_3$): δ 0.98 (br t, 3 H), 1.1–1.9 (m, 4 H), 2.95 (s, 6 H), 3.64 (s, 2 H), 3.95 (t, 2 H, J = 6 Hz), 6.7–7.4 (AB system, J_{AB} = 10 Hz, $\Delta\delta$ 0.35 ppm, 4 H).

18 (90 MHz, $CDCl_3$): δ 0.99 (t, J = 6.7 Hz, 3 H), 1.3–2 (m, 4 H), 3.54 (s, 2 H), 4 (t, J = 6.7 Hz, 2 H), 5.3–6.3 (2 H), 6.8–7.4 (m, 4 H).

27 (60 MHz, $CDCl_3$): δ 4.50 (s, 2 H), 6.15–7 (2 H), 6.70–7.50 (m, 5 H).

29 (60 MHz, $CDCl_3$): δ 0.97 (br t, 3 H), 1.2–2 (m, 4 H), 2.85–3.1 (m, 4 H), 3.95 (t, J = 6.5 Hz, 2 H), 6.70–7.28 (AB system, J_{AB} = 10 Hz, $\Delta\delta$ 0.35 ppm, 4 H).

Table III. Inhibition. Effects of Hydrophobic Substituents of Phenylacetamide Derivatives

compd	chemical structure	K_I (μM)	mp (lit.), °C	prepn method	structure assignment
1		140	159-160 (159-160[b])	A	
13		70	188.5-189 (188-189[c])	1	
14		80	194-194.5 (184[d])	1	
15		80	144-145	1	
16		25	191-192	1	
17		7	189.5-190 (186-187[e])	1	
18		15	112.5-113	K	*a*
19		6	180-180.5 (176-178[e])	1	
20		1.2	185-186 (149-150[e])	1	
21		2	199-200 (199-200[f])	1	
22		2	214-216	J	
23		1	J		
24		170	175-176 (180[g])	C	

[a] NMR data are given in the Experimental Section. [b] Danis, C. H.; Carmack, M. *J. Org. Chem.* 1947, *12*, 76-78. [c] Burger, A.; Avakian, S. *Ibid.* 1940, *5*, 606-609. [d] Werner, A. *Ann. der Chemie*, 1902, *322*, 151-152. [e] Buu Hoi, N. P.; Lambelin, G.; Lepoivre, C.; Gillet, C.; Thiriaux, J. *Chem. Abstr.* 1967, *66*, 18607a. [f] Beilstein, 4. Auflage, Drittes Ergänzungswerk, Band X (1), p. 436. [g] Sasaki, S.; Eguchi, S.; Toru, T. *Bull. Chem. Soc. Jpn.* 1968, *41*, 238-240.

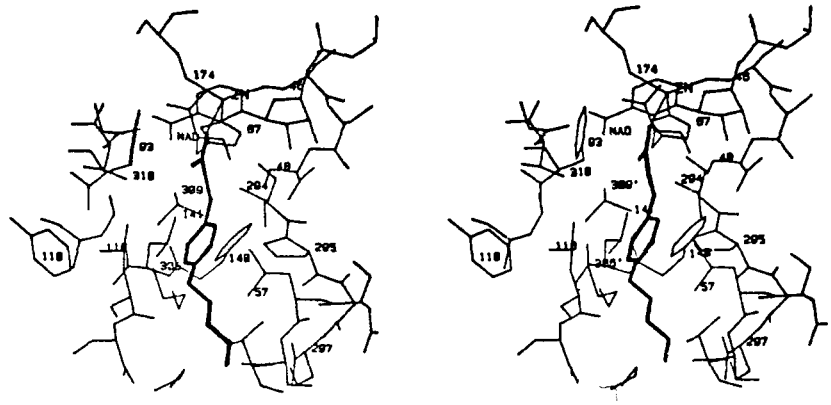


Figure 3. Stereodiagram of the bound 3-(*p*-butoxyphenyl)propionamide to liver alcohol dehydrogenase–NADH complex. The inhibitor structure is drawn in darker lines. The oxygen atom of the amide group acts as fourth ligand to the zinc ion (ZN) and the nitrogen is oriented toward the imidazole ring of His-67. (obtained as in Figure 1). For the binding of formamide-derived inhibitors replace the methylene group by a NH group oriented toward the hydroxyl group of Ser-48. This stereodiagram was obtained by model building with the Interactive Graphic Display.

30 (60 MHz, $CDCl_3$–CD_3OD): δ 0.96 (br t, 3 H), 1.2–1.9 (m, 4 H), 2.20–3.04 (m, 4 H), 3.91 (t, J = 6 Hz, 2 H), 6.70–7.75 (AB system, J_{AB} = 9 Hz, $\Delta\delta$ 0.32 ppm, 4 H).

32 (90 MHz, $CDCl_3$): δ 4.29 (d, J = 6 Hz, 2 H), 6.7–7.2 (1 H), 7.21 (s, 5 H), 8.04 (s, 1 H).

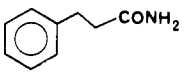
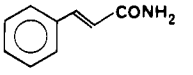
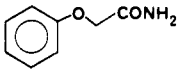
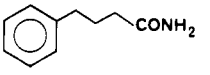
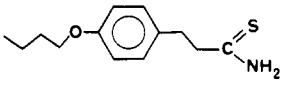
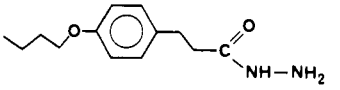
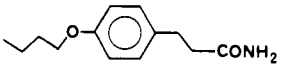
33 (60 MHz, CD_3OD): δ 4.32 (s, 2 H), 6.7–7.3 (AB system, J_{AB} = 8 Hz, $\Delta\delta$ 0.4 ppm, 4 H), 8.2 (s, 1 H).

34 ($CDCl_3$): δ 0.99 (br t, 3 H), 1.2–2 (m, 4 H), 3.97 (t, J = 6.7 Hz, 2 H), 4.42 (d, J = 6 Hz, 2 H), 6–6.4 (1 H), 6.8–7.4 (AB system, J_{AB} = 8 Hz, $\Delta\delta$ 0.36 ppm, 4 H), 8.26 (s, 1 H).

37 (60 MHz, CD_3COCD_3): δ 4.4 (d, J = 6 Hz, 2 H), 5.35 (s, 2 H), 6.82–7.35 (AB system, J_{AB} = 9 Hz, $\Delta\delta$ 0.3 ppm, 4 H), 7.39 (s, 5 H), 8.23 (s, 1 H).

38 (60 MHz, $CDCl_3$): δ 0.98 (br t, 3 H), 1.2–2 (m, 4 H), 3.95 (t, J

Table IV. 3-Phenylpropionamide Derivatives and 3-Phenylpropionamide-like Inhibitors

compd	chemical structure	K_I (μM)	mp (lit.), °C	prepn method	structure assignment
25		25	95-96 (101.5-102[b])	C	
26		11	146-147 (147[c])	C	
27		20	100-101 (101.5[d])	U	*a*
28		12	81.5-82 (83-84[e])	C	
29		5.5	103-105	N	*a*
30		200	132.5-133	M	*a*
31		0.12	131-132	J	

[a] NMR data are given in the Experimental Section. [b] Baker, W.; Lapworth, A. *J. Chem. Soc.* 1924, *125*, 2333-2338. [c] Kobayashi, S. *Chem. Zentr.* 1928, *I*, 1930-1931. [d] Fritzsche, P. *J. Prakt. Chem.* 1879, *20*, 267-300. [e] Blicke, F. F.; Sheets, D. G. *J. Am. Chem. Soc.* 1948, *70*, 3768-3770.

= 6 Hz, 2 H), 4.42 (d, J = 6 Hz, 1 H), 5.8-6.2 (1 H), 6.7-7.45 (m, 4 H), 8.26 (s, 1 H).

40 (60 MHz, $CDCl_3$): δ 1.92 (s, 3 H), 4.3 (d, J = 3.5 Hz, 2 H), 6.6-7 (1 H)8 7.19 (s, 5 H).

41 (60 MHz, $CDCl_3$): δ 4.48 (d, J = 6 Hz, 2 H), 7.36 (s, 5 H).

42 (60 MHz, $CDCl_3$): δ 2.76 and 2.82 (J = 3.5 Hz, 3 H), 4.38 and 4.52 (J = 8 Hz, 2 H), 7.26 (5 H), 8.16 and 8.28 (1 H).

50 (90 MHz, $CDCl_3$): δ 1.3-1.9 (m, 10 H), 2.3 (t, J = 9 Hz, 2 H), 3.45 (s, 2 H), 3.95 (t, J = 6 Hz, 2 H), 7.03 (AB system, J_{AB} = 9 Hz, Δδ 0.35 ppm, 4 H).

55 (60 MHz, D_2O): δ 3.7-4.2 (br, 7 H), 4.5 (s, 2 H), 7.35 (AB system, $J \sim 4$ Hz, 4 H).

56 (60 MHz, D_2O): δ 1.3-2 (br, 6 H), 2.2-2.5 (br, 2 H), 3.6-4.2 (br, 7 H), 7.2 (AB system, $J \sim 7$ Hz, 4 H).

Model Building. In the time course of this work, the design and the interpretation were made using three types of representation of the enzyme structure. The first one was a Kendrew model of a diamond lattice fitted to the substrate binding site of alcohol dehydrogenase.[12] The second one was a Kendrew model of the substrate binding site built according refined X-ray coordinates. The final model building was made on the Interactive Graphic Display System (Vector General 3400) in Uppsala (Sweden).

The inhibitor molecules were fitted within the refined holoenzyme structure and placed in such way that the maximum hydrophilic and hydrophobic interactions with the protein residues were attained. Except in the case of hydrogen bonds for which a distance of 2.5 Å was choosen, no contacts closer than 3 Å were accepted. The atom liganded to the catalytic zinc ion was placed at the position found for the oxygen atom of the ligand in the structure determinations of ternary enzyme–(NAD^+ or NADH)–ligand complexes.[8-10]

Each inhibitor molecule was built according to the published values of bond distances and bond angles. Their best positions were determined through translation operations and/or torsion angle changes using the FRODO program.[21,22]

Results

All the compounds described here were tested with alcohol dehydrogenase from horse liver, and were inhibitors competitive in respect to acetaldehyde with the exception of the amide–acid **50** which is a noncompetitive inhibitor. The results are presented in Tables I to VIII as K_i determined according to Lineweaver–Burk.[20]

Discussion

Structural Information. The substrate binding site of alcohol dehydrogenase appears as a 20 Å long channel, going from the solution to the catalytic center where the catalytic zinc ion is located.[23] The hydrophilic zone in the vicinity of the zinc ion is aligned with the *Re* side of the nicotinamide ring, Cys-46, His-67, Ser-48, and Phe-93 side chains. Cys-46 and His-67 side chains are two zinc ligands. Further a narrow space between Leu-57, Leu-116, Leu-141, Val-294, and Ileu-318 side chains leads to a zone where the side chains of residues from both subunits are found: Phe-110, Met-306′,* [LADH is a dimeric enzyme; the sign prime holds for the second subunit], Leu-309′. This part is essentially hydrophobic. The substrate binding site then extends to some polar residues: main chain carbonyl group of 116 and

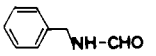
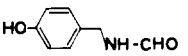
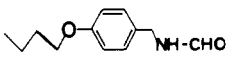
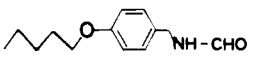
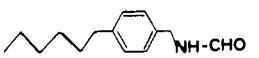
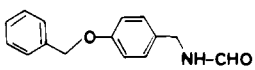
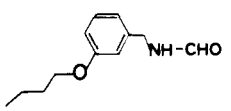
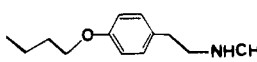
(21) Jones, T. A. *J. Appl. Crystallogr.* **1978**, *11*, 268-272.

(22) Jones, T. A. "Computational Crystallography"; Sayre, D., Ed.; Oxford University Press: Oxford, 1982; pp 303-317.

(23) Eklund, H.; Nordström, B.; Zeppezauer, E.; Söderlund, G.; Ohlsson, I.; Boiwe, T.; Söderberg, B. O.; Tapia, O.; Bränden, C. I.; Åkeson, Å. *J. Mol. Biol.* **1976**, *102*, 27-59.

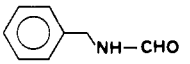
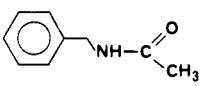
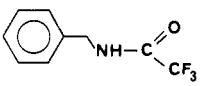
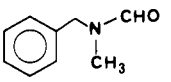
(24) Sund, H.; Theorell, H. "The Enzymes", 2nd ed.; Boyer, P. D., Lardy, H., Myrbäck, K., Eds.; Academic Press: New York, 1963; Vol. 7, pp 25-83.

Table V. Inhibitors of Alcohol Dehydrogenase Containing the Formamide Function

compd	chemical structure	K_I (μM)	mp (lit.), °C	prepn method	structure assignment
32		3	56.5–57 (59.8–60.4[b])	N	*a*
33		1.8	127–128 (129–130[c])	R	*a*
34		0.04	56–57	R	*a*
35		0.03	62–62.5	R	
36		0.026	53–54	T	
37		0.015	117–118	R	*a*
38		0.1	46–47	R	*a*
39		2	53–54	R	

[a] NMR data are given in the Experimental Section. [b] Buehler, C. A.; Mackenzie, C. A. *J. Am. Chem. Soc.* 1937, *59*, 421–422. [c] Sekiya, M.; Yanaihara, N.; Masui, T., *Chem. Pharm. Bull.* 1961, *9*, 945–948.

Table VI. Inhibition. Effects of the Substitution of the Amide Group

compd	chemical structure	K_I (μM)	mp (lit.)	prepn method	structure assignment
32		3	56.5–57 (59.8–60.4[b])	N	*a*
40		1000	61–62 (61[c])	G	*a*
41		300	70.5–71 (72–75[d])	H	*a*
42		25	([e])	I	*a*

[a] NMR data are given in the Experimental Section. [b] Buehler, C. A.; Mackenzie, C. A. *J. Am. Chem. Soc.* 1937, *59*, 421–422. [c] Erickson, J. L. E. *Chem. Ber.* 1926, *59B*, 2665–2668. [d] Meresaar, U.; Bratt, L. *Acta Chem. Scand.* 1974, *28*, 715–722. [e] Franconi, C. *Z. Electrochem.* 1961, *65*, 645–648.

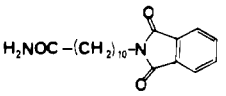
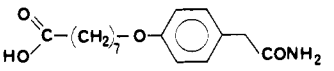
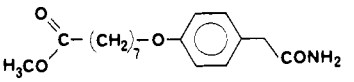
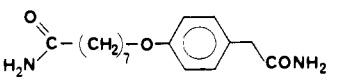
the side chains of Ser-117, Ser-310′, Arg-312′, and Glu 384′ and to the solution. The geometry and the distribution of amino acids along the substrate binding site of alcohol dehydrogenase provide the molecular explanation for the broad substrate specificity of the enzyme.[24]

The results will be discussed using the model building interpretations from the Interactive Graphic Display. The order of discussion of the results will be done on the basis of amide skeleton: phenylacetamide, 3-phenylpropionamide, and formamide.

Derivatives of Phenylacetamide. Two binding modes of the amide group seem possible: with the oxygen acting as fourth ligand to the zinc ion, the NH_2 group can be oriented toward the ring nitrogen of the dihydronicotinamide ring of NADH or toward the nitrogen of the amide of NADH (Figure 1). In both cases, the positions of the oxygen atom and the phenyl ring are identical. Substitution of the NH_2 group of the inhibitor will create steric conflict with the phenyl ring of Phe-93 or with the dihydronicotinamide ring of NADH (molecules **2** and **3**) (Table I). In either binding mode, the methylene group of phenyl acetamide is positioned in a crowded volume. It is sandwiched between Ser-48 and Phe-93 side chains at about 3 Å to the nearest residue in one case and about 3 Å to the Ser-48 side chain and to the dihydronicotinamide ring of NADH in the other case. In any binding mode, substitution at the methylene group will be unfavorable to the binding as shown by molecules **4** and **5** (Table I).

The phenyl ring is surrounded by the side chains of Leu-116, Leu-141, Val-294, and Ile-318, the shortest distance being 3–3.5 Å. Para substitution is favored compared to ortho or meta substitution as confirmed by value of the inhibition constant of molecules **7**, **8**, and **9** (Table II).

Table VII. Bifunctional Inhibitor Molecules

compd	chemical structure	K_I (μM)	mp (lit.), °C	prepn method	structure assignment
43	$H_2NOC-(CH_2)_{10}-CONH_2$	0.9	189-190 (189[b])	C	
44	$H_2NOC-(CH_2)_{11}-CONH_2$	0.7	173-174	C	
45	$H_2NOC-(CH_2)_{12}-CONH_2$	0.25	186-189 (186-187[c])	C	
46	$H_2NOC-(CH_2)_{14}-CONH_2$	0.18	181-183	C	
47	$CH_3-(CH_2)_{14}-CONH_2$	6	99-100 (107[d])	C	
48	$H_2NOC-(CH_2)_{10}-NHC(=NH)NH_2$ $\frac{1}{2}H_2SO_4$	0.12	100-102	P	
49		0.65	154-155	Q	
50		100 noncompetitive	150-151	L	*a*
51		2.5	144-145	L	
52		0.3	270-275	L	
53	$H_2NOC-(CH_2)_{16}-NHCHO$	0.43	109-111	O	
54	$OHCHN-(CH_2)_{12}-NHCHO$	0.058	100-100.5	F	

[a] NMR data are given in the Experimental Section. [b] Barnicoat, C. R. *J. Chem. Soc.* 1927, 2926-2929. [c] Garmaise, D. L.; Kay, R. W.; Gaudry, R.; Baker, H. A.; McKay, A. F. *Can. J. Chem.* 1961, *39*, 1493-1501. [d] Ralston, A. W., Hoerr, C. W.; Pool, W. O. *J. Org. Chem.* 1943, *8*, 473-488.

The hydrophobic environment in the proximity of the para position of the aromatic ring is provided by the side chains of Leu-116 and Leu-57 (Figure 2). This agrees with the increased inhibitory power of the *p*-halogenosubstituted phenylacetamides **9**, **10**, **11**, and **12**. The more bulky and less polar iodine substitutent leads to the best inhibition. The para substitution with various hydrophobic groups of increasing size leads to inhibitor molecules with K_I values approaching 1 μM: molecules **13** to **23** (Table III). The phenyl group can be replaced by another hydrophobic skeleton, for instance, 1-adamantyl as in amide **24** (Table III).

Derivatives of 3-Phenylpropionamide (25, Table IV). Maintaining the oxygen atom in the position deduced from X-ray structure determinations, three possible configurations are found for the amide group of 3-phenylpropionamide. NH_2 of the amide group can be at a 3.0-Å distance of the imidazole ring of His-67 (Figure 3) or pointing toward the C-6 position of the dihydronicotinamide ring of NADH at a distance of 3.1 Å or placed at about 3.0 Å of C-4 position of dihydronicotinamide ring of NADH and of the C_2 carbon atom of the Phe-93 side chain (not shown). In all three cases, the trans double bond of cinnamic amide **26** does not bring any new constraint. The replacement of a methylene by an oxygen as in **27** is hardly detectable in term of binding differences.

The additional methylene group in 3-phenylpropionamide (**25**) compared with phenylacetamide (**1**) introduces a new torsion angle. This increases the flexibility of the molecule, displaces the phenyl ring in the substrate binding site, away from the zinc atom, and ideally places the para position of the ring for further substitution. The binding of the inhibitor **25** is favored by an order of magnitude compared with molecule **1**.

The *p*-butoxy chain of molecule **31** extends in the hydrophobic binding site, and a possible binding mode is presented in Figure 3. It is worth mentioning the two orders of magnitude improvement in the K_I value for molecule **31** compared with the K_I value of molecule **25** which are not observed in the phenylacetamide serie (**1** and **17**).

Increasing the size of the amide group has a negative effect. Molecule **30** becomes equivalent to the phenylacetamide **2** with respect to the K_I values. The hydrazide and *N*-methyl derivatives are not accepted for steric reasons.

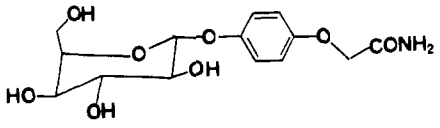
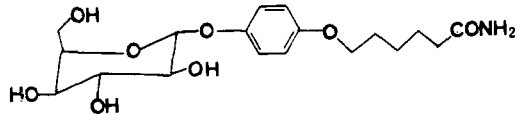
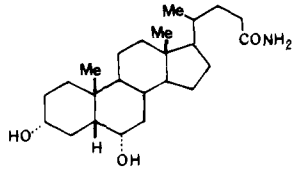
The less favorable binding of thioamide **29** may be due to the increase of the van der Waals radius of sulfur and of the C=S distance compared with those of oxygen or to the less favorable zinc ion–thioamide interaction. No value for the complex formation of thioamide with zinc ion could be found in the literature.

Addition of a third methylene group between amide and phenyl groups (molecule **28**) slightly improves the K_I value. However, this should better be interpreted as an expected improvement of inhibition power due to an increased hydrophobicity than as a more favorable binding of that type of skeleton. In other words, a *p*-butoxy substituent on such a molecule would not necessarily lead to a better inhibitor than molecule **31**. This hypothesis is consistent with what happens in the formamide series for molecules **34** and **39** (Table V).

Derivatives of Formamide. Although the amide function as zinc ligand proved to be efficient, it was clear that the NH_2 group did not contribute to the binding in any of the former cases.

In order to improve the inhibitory properties, we anticipated the role of the Ser-48 side chain in binding formamide. Model building shows that with such inhibitors the carbonyl group can act as a zinc ligand while the formamide nitrogen is at a hydrogen

Table VIII. Inhibitor Molecules of Increased Water Solubility

compd	chemical structure	K_I (μM)	mp, °C	prepn method	structure assignment
55		2000	216-218	U	*a*
56		12	82-83	U	*a*
57		4.4	268-270	E	

[a] NMR data are given in the Experimental Section.

bond distance (2.6 Å) to the oxygen of the Ser-48 side chain (see Figure 3 and replace the methylene of phenylpropionamide with a NH group). The other possible binding mode where the nitrogen would be liganded to the zinc ion is not allowed for steric reasons. Molecule **32** exhibits improved inhibitory properties compared with 3-phenylpropionamide (**25**). The phenyl position is well suited for para substitution, as in the 3-phenylpropionamide case. Hydrophobic substituents in the para position (molecules **34**, **35**, and **36**) (Table V) indeed improve the binding properties and the inhibition power. The second aromatic ring in molecule **37** is found in the vicinity and at van der Waals distance of the Phe-110 side chain. This inhibitor is therefore very tightly bound in the substrate binding site through the carbonyl oxygen–zinc ion interaction, the hydrogen bond to Ser-48 side chain, and the van der Waals contacts of the first aromatic ring to the hydrophobic residues and of the second aromatic ring to Phe-110 side chain.

In a slightly different binding mode of the formamide, the phenolic hydrogen of molecule **33** could interact with the main-chain carbonyl oxygen of Leu-116. Meta substitution is less favorable although allowed for a flexible hydrophobic chain (molecule **38**) (Table V).

Methyl substitution at the nitrogen atom as in formamide **42** (Table VI) breaks the hydrogen bond to the Ser-48 and requires repositioning of the Ser-48 side chain in order to release the steric strain. This is expressed in the K_I value which is reduced by an order of magnitude and which becomes similar to that of **27**.

Acetamide **40** and trifluoroacetamide **41** become too crowded and give raise to steric interaction with the dihydronicotinamide ring (Table VI). This situation could only be released with a different binding mode which is much less favorable as in the case of *N*-phenylurea **6** (Table I).

Bifunctional Inhibitors (Table VII). At the bottom of the substrate binding site, close to the outer solution, some polar residues are found. They could provide additional binding interactions and some bifunctional inhibitors were prepared. This polar character of the binding site explains the better binding of molecule **46** compared with molecule **47**.

Examination of the structure tells that about 10 methylene groups in an extended alkyl chain would fill the entire channel. Indeed the inhibitory properties of series **43**, **44**, **45**, and **46** are in good agreement with this observation. The smallest inhibition constant is observed with molecule **54**. This improvement of the binding constant is related to the presence of the formamide function as a zinc ligand.

Conclusion

The basic structure for the good inhibitors designed in this study has a carbonyl group liganded to the zinc ion through the oxygen atom, a spacer equivalent to two methylene groups, and a phenyl ring. This aromatic group is thus surrounded by hydrophobic side chains of Val-294, Ile-318, Leu-116, and Leu-57 at van der Waals distances. The formamide nitrogen is close to the mobile side chain of Ser-48, possibly forming a hydrogen bond. In the para position of the phenyl ring, any hydrophobic substituent increases the inhibitory properties.

It is of interest to recall the behavior of aromatic substrates of alcohol dehydrogenase. *p*-Bromobenzyl alcohol induces a deviation from the ordered bi–bi mechanism.[25] The molecular explanation to this fact is that such a substrate of hydrophobic character may bind to the apoenzyme whereas ethanol cannot. More striking is *p*-dimethylaminocinnamaldehyde (DACA) which forms a transient ternary complex LADH–NADH–DACA.[26] A simulated transient complex was described with tetrahydronicotinamide–adenine dinucleotide[27] and studied crystallographically.[9] This substrate is structurally similar to molecule **26** and probably binds in a closely related way. Thus the stability of such complexes may be related to the role played by the phenyl ring in the basic structure discussed above.

Our aim was to reach very small inhibition constants, and actually some of the molecules presented here bind very tightly to the LADH–NADH complex. One drawback is the hydrophobic character of these molecules which is dictated by the nature of the substrate binding site of the enzyme. The amides **55**, **56**, and **57** were prepared to provide more water-soluble compounds (Table VIII). The hydroxyl groups at C-3 and C-6 in the steroidal amide **57** might form hydrogen bonds at the polar area of the substrate binding site. The inhibitory properties of molecule **57** correlates well with the fact that 5β-cholestane 3α,7α,12α,26-tetraol is a substrate of the enzyme.[28-30]

(25) Bernhard, S. A.; Dunn, M. F.; Luisi, P. L.; Schack, P. *Biochemistry* **1970**, *9*, 185–192.
(26) Dunn, M. F.; Hutchinson, J. S. *Biochemistry* **1973**, *12*, 4882–4892.
(27) Dunn, M. F.; Biellmann, J. F.; Branlant, G. *Biochemistry* **1975**, *14*, 3176–3182.
(28) Okuda, K.; Takigawa, N. *Biochim. Biophys. Acta* **1970**, *220*, 141–148.
(29) Björkhem, I.; Jörnvall, H.; Zeppezauer, E. *Biochem. Biophys. Res. Commun.* **1973**, *52*, 413–420.
(30) Björkhem, I.; Jörnvall, H.; Åkeson, Å. *Biochem. Biophys. Res. Commun.* **1974**, *57*, 870–875.

Acknowledgment. We are indebted to Professor C. I. Bränden and Dr. H. Eklund (Uppsala, Sweden) for providing us coordinates and extensive structural information, and all facilities in using the Graphic Display. Our thanks go to Professor H. Dutler (Basel, Switzerland) and C. I. Bränden for the diamond lattice adapted to the substrate site of the enzyme. This work was supported by grants from the Institute de Recherches Scientifiques Economiques et Sociales sur les Boissons (I.R.E.B.), from the Fondation pour la Recherche Médicale Francaise, and from the P.I.R.M.E.D. (ASP-CNRS No. 2280).

Registry No. 1, 103-81-1; **2**, 89789-99-1; **3**, 89790-00-1; **4**, 1125-70-8; **5**, 10255-95-5; **6**, 64-10-8; **7**, 10268-06-1; **8**, 58357-84-9; **9**, 20101-92-2; **10**, 332-29-6; **11**, 74860-13-2; **12**, 84863-81-0; **13**, 6343-93-7; **14**, 40784-91-6; **15**, 89790-01-2; **16**, 89790-02-3; **17**, 3413-59-0; **18**, 89790-03-4; **19**, 14442-83-2; **20**, 5100-05-0; **21**, 84199-13-3; **22**, 89790-04-5; **23**, 89790-05-6; **24**, 19026-73-4; **25**, 102-93-2; **26**, 621-79-4; **27**, 621-88-5; **28**, 1199-98-0; **29**, 89790-06-7; **30**, 89790-07-8; **31**, 89790-08-9; **32**, 6343-54-0; **33**, 86386-69-8; **34**, 87578-63-0; **35**, 89790-09-0; **36**, 89790-10-3; **37**, 89790-11-4; **38**, 89790-12-5; **39**, 89790-13-6; **40**, 588-46-5; **41**, 7387-69-1; **42**, 17105-71-4; **43**, 6224-99-3; **44**, 89790-14-7; **45**, 61382-93-2; **46**, 89790-15-8; **47**, 629-54-9; **48**, 89790-17-0; **49**, 89790-18-1; **50**, 89790-19-2; **51**, 89790-20-5; **52**, 89790-21-6; **53**, 89790-22-7; **54**, 87053-07-4; **55**, 89790-23-8; **56**, 89790-24-9; **57**, 89790-25-0; phenylacetonitrile, 140-29-4; *p*-pentoxyphenylacetonitrile, 50690-55-6; 3-phenylpropionic acid, 501-52-0; *p*-butoxyphenylacetic acid, 4547-57-3; methyl *p*-butoxyphenylacetate, 29056-06-2; benzylamine, 100-46-9; formic acid, 64-18-6; acetic anhydride, 108-24-7; trifluoroacetic anhydride, 407-25-0; *p*-hydroxyphenylacetonitrile, 14191-95-8; *p*-hydroxyphenylacetic acid, 156-38-7; 3-(*p*-butoxyphenyl)propanoic acid, 3243-41-2; *m*-hydroxybenzylic alcohol, 621-37-4; methyl 8-hydroxyoctanoate, 20257-95-8; *p*-hydroxybenzaldehyde, 123-08-0; *p*-butoxybenzaldehyde, 5736-88-9; cyanomethyltriphenylphosphonium chloride, 4336-70-3; *p*-butoxycinnamonitrile, 89790-26-1; 3-(*p*-butoxyphenyl)propionitrile, 89790-27-2; 11-aminoundecanoic acid, 2432-99-7; *N*-formyl-11-aminoundecanoic acid, 3611-31-2; methyl-11-aminoundecanoic acid, 28691-27-2; *p*-butoxybenzonitrile, 38746-93-9; glycine, 56-40-6; *N*-formylglycine, 2491-15-8; propylamine, 107-10-8; 1-bromopentane, 110-53-2; *n*-pentyltriphenylphosphonium bromide, 21406-61-1; 4-cyanobenzaldehyde, 105-07-7; 1-(*p*-cyanophenyl)hexene-1, 89790-28-3; 1-(*p*-cyanophenyl)hexane, 29147-95-3; *p*-arbutin, 497-76-7; iodoacetamide, 144-48-9; *m*-butoxybenzyl alcohol, 30609-21-3; *m*-butoxybenzoyl chloride, 89790-29-4; *m*-butoxyphenylacetnitrile, 74205-57-5; methyl 8-hydroxyoctanoate tosylate, 89790-30-7; *p*-(7-methoxycarbonylheptamethyleneoxy)phenylacetamide, 89790-31-8; (triphenylphosphoranylidene)acetonitrile, 16640-68-9; triphenylphosphine, 603-35-0; chloroacetonitrile, 107-14-2; methyl 11-guanidinoundecanoate hemisulfate, 89790-33-0; alcohol dehydrogenase, 9031-72-5.

Communications to the Editor

General Synthesis of Pentacyclic Quassinoids

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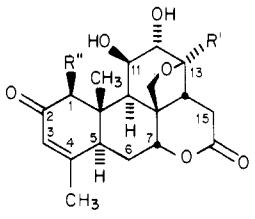
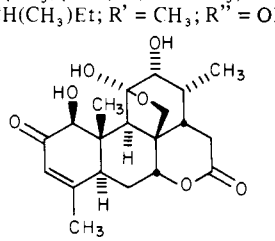
Received January 4, 1984
Revised Manuscript Received March 12, 1984

The family of bitter principles known as quassinoids includes compounds displaying antileukemic, antineoplastic, antimalarial, and even insecticidal and antifeedant properties.[1] Among these, only pentacyclic quassinoids, which possess A-ring enone functionality and a bridging ether to C11 or C13 (e.g. **1**–**4**), are of



1, R = COCH=C(CH$_3$)-*i*-Pr; R' = CO$_2$CH$_3$; R'' = H
2, R = COC(CH$_3$)(OAc)Et; R' = CH$_3$; R'' = OH
3, R = COCH(CH$_3$)Et; R' = CH$_3$; R'' = OH



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medicinal interest. Here we describe a series of stereoselective annelations as well as a novel isomerization of C13- to C11-bridged intermediates, which for the first time permits access to both main classes of pentacyclic quassinoids.[2]

Much of the carbon skeleton was assembled in a single conjugate addition–enolate trapping using *trans*-1-iodo-3-(benzyloxy)-1-pentene (**5**)[3] and 4-prenyl-3-methyl-2-cyclohexenone (**6**) (Scheme I).[4] Modification of Noyori's organocopper-based procedure[5] generated a reactive lithium enolate suitable for in situ alkylation. Thus **5** was metalated (*n*-BuLi, 1.2 equiv, Et$_2$O) then treated with CuI–Bu$_3$P (2 equiv) at −70 °C. After addition of **6** (1 equiv), the mixture was warmed to −35 °C (1.5 h) and recooled, and more *n*-BuLi (1.2 equiv) was added to transform the obligatory Cu(I) enolate to (*n*-BuCu)$_x$ and a lithium enolate. Subsequent addition of (EtO)$_2$POCl–Et$_3$N (4 equiv) furnished triene **7** as a colorless oil in 87% yield (20-g scale).[6] This improved coupling should find widespread use when both the nucleophile and electrophile must not be wasted. The stereochemistry shown in **7**, anticipated from related cuprate additions,[7] was later confirmed by X-ray diffraction analysis.[8]

Reductive cleavage of **7** (Li, NH$_3$, *t*-BuOH) then oxidation of **8** afforded enone **9** (75%). Stereoselective conjugate addition of ethyl cyanoacetate to **9** furnished **10** (55%), establishing the correct configuration at C9 (vide infra) for the quassinoids. Ozonolysis and cyclization of **10** (Me$_2$S–NaHCO$_3$) formed alcohol **11**, as judged by the C7 methine resonance in its acetate ester **12** (δ 5.36, J = 11.1, 4.8 Hz). Cyclization of **11** (LiOEt, EtOH, room tem-

(1) For a review, see: Polonsky, J. *Fortschr. Chem. Org. Naturst.* **1973**, *30*, 101.

(2) For leading references to synthetic efforts at quassinoid total synthesis, see: Grieco, P. A.; Lis, R.; Ferrino, S.; Jaw, J. Y. *J. Org. Chem.* **1982**, *47*, 601.

(3) Prepared from *trans*-1-chloro-1-penten-3-one according to the procedure of: Corey, E. J.; Beames, D. J. *J. Am. Chem. Soc.* **1972**, *94*, 7210.

(4) Danheiser, R. L.; Stork, G. *J. Org. Chem.* **1973**, *38*, 1775.

(5) Suzuki, M.; Suzuki, T.; Kawagishi, T.; Noyori, R. *Tetrahedron Lett.* **1980**, *21*, 1247.

(6) Satisfactory IR, NMR, and mass spectral data have been obtained for all new substances described.

(7) (a) Wege, P. M.; Clark, R. D.; Heathcock, C. H. *J. Org. Chem.* **1976**, *41*, 3144. (b) Ziegler, F. E.; Reid, G. R.; Studt, W. L.; Wender, P. A. *Ibid.* **1977**, *42*, 1991.

(8) Batt, D. G. Ph.D. Thesis, Cornell University, Ithaca, NY, 1981.

0002-7863/84/1506-3353$01.50/0