

3-Substituted Pyrazole Derivatives as Inhibitors and Inactivators of Liver Alcohol Dehydrogenase

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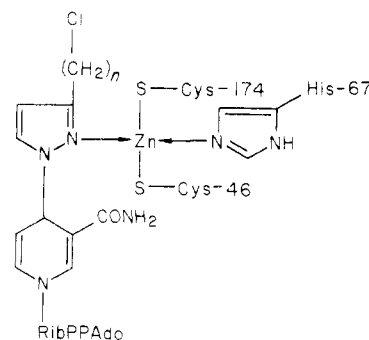
3-Substituted pyrazoles, HOCH₂ (1), HOCH₂CH₂ (2), HOCH₂CH₂CH₂ (3), ClCH₂ (4), ClCH₂CH₂ (5), ClCH₂CH₂CH₂ (6), and CH₃CO (7), were synthesized and evaluated *in vitro* on horse liver alcohol dehydrogenase for their potential as inhibitors of ethanol metabolism. 1 to 6 bound to the enzyme-NAD⁺ complex with dissociation constants of 40 to 200 μM, much higher than the constants for the corresponding 4-substituted pyrazoles, but with the same absorption maximum at 295 nm. 4 inactivated the enzyme within a few minutes, but NAD⁺ protected against reaction, and 4 nonspecifically alkylated many sulfur atoms in the protein. The isomer, 4-(chloromethyl)pyrazole, behaved similarly. 5 and 6 strongly inhibited the enzyme in the presence of NAD⁺, due to formation of the slowly dissociable (10⁻³ s⁻¹) enzyme-NAD⁺-pyrazole complex, but did not irreversibly inactivate the enzyme. 7 inhibits the enzyme weakly (*K_D* = 5 mM). It appears that the 3-substituted pyrazoles bind to the enzyme-NAD⁺ complex with the reactive functional group improperly positioned for specific irreversible reaction.

Liver alcohol dehydrogenase (EC 1.1.1.1) catalyzes the first step in alcohol metabolism and would be a rational target for inhibiting alcohol metabolism.^{1,2} Prevention of poisoning by methanol and of damaging effects of ethanol metabolism are potential applications of inhibitors of alcohol dehydrogenase. Pyrazole and some of its 4-substituted derivatives are known to bind especially tightly to the enzyme-NAD⁺ complex with inhibition constants as low as 0.8 nM.^{3,4} Pyrazole and its 4-methyl, iodo, and bromo derivatives are potent inhibitors of ethanol metabolism *in vivo*.⁵⁻⁹ Unfortunately, pyrazole is itself toxic and may not be useful for long-term treatment of humans.⁹

Recently, diazonium-1*H*-tetrazole was found to inactivate the horse liver enzyme *in vitro* in an almost stoichiometric reaction that modified cysteine residue 174, a ligand for the active-site zinc. The specificity of the reaction could be due to the ligation of the tetrazole ring to the zinc (in a manner similar to that proposed for the binding of pyrazole¹⁰) and the orientation of the diazonium group toward the sulfur of Cys-174.¹¹ Inspection of models of zinc-pyrazole complexes and of the three-dimensional structure of liver alcohol dehydrogenase^{12,13} suggested that substituents in the 3 position, but not those in the 4 position, could be oriented toward the sulfur, as shown in Scheme I. When *n* is from 1 to 3, reaction seems possible. If these substituents were chemically reactive, the pyrazoles could be active-site-directed reagents that would alkylate and irreversibly inactivate the enzyme specifically in the presence of NAD⁺; they might thus be more effective and less toxic *in vivo* than reversible inhibitors, such as 4-substituted pyrazoles. In this work, a series of new 3-substituted pyrazole derivatives was synthesized and evaluated as potential specific inhibitors or inactivators of alcohol dehydrogenase. Our studies also permit a comparison of the inhibitory potency of a series of 3- and 4-substituted pyrazoles. Only the 3- and 4-methylpyrazoles have been compared previously.^{3,4}

Chemistry. The 3-(ω-hydroxyalkyl)pyrazoles (Table I) were prepared by the method of Jones¹⁴ from diazomethane and the appropriate alkynol and purified by distillation *in vacuo*. Small amounts of the 4-substituted derivatives were also produced. The NMR spectra showed a pair of doublets at about δ 6.1 and 7.5 for the ring hydrogens and signals for methylene hydrogens at δ 3.8 to 3.4 (CH₂O), 2.6 (pyrazole CH₂), and 1.8 to 2.1 (other CH₂). Reference samples of some corresponding 4-substituted derivatives showed singlets at δ 7.4 for the ring hydrogens, and on the basis of this signal it was estimated that the 3-(hydroxyalkyl)pyrazoles could have contained about 10% of the 4-substituted derivatives. The 3-(ω-chloroalkyl)pyrazoles were prepared with SOCl₂ and the hydrochloride

Scheme I



salts of the corresponding hydroxy compounds.¹⁴ The ring hydrogen doublets appeared at δ 8.1 and 6.5 for the 3-substituted derivatives and the ring hydrogens' singlet at δ 8.1 for 4-substituted derivatives; the 3-substituted derivatives appeared to be free of contamination by the 4-substituted derivatives.

Biochemical Results and Discussion. Pyrazole reacts with enzyme and NAD⁺, releasing a proton (at pH 7) and forming a complex with an absorbance maximum at 295 nm, and it has been postulated that pyrazole forms a covalent adduct or charge-transfer complex with NAD⁺, as shown in Scheme I.¹⁰ The binding of pyrazole derivatives thus can be measured spectrophotometrically, usually at 300 nm in order to avoid working with solutions with high absorbance due to the high concentration of enzyme. The results in Figure 1 show that the 3- and 4-substituted pyrazoles form absorbing complexes, which are tight enough in the case of pyrazole and 4-(2-hydroxyethyl)pyrazole to allow one to determine by titration the concentration of pyrazole. For the 3-substituted pyrazoles, the complex is weaker and the titration point is not visible, but using such a method it is possible to determine dissociation constants for the pyrazole derivatives. [Figure 1 also shows that 3-(2-hydroxyethyl)pyrazole contains less than 10% of any contaminating 4-substituted derivative, as estimated by extrapolating the biphasic curve to zero enzyme concentration.] The results in Table II show that the 3-substituted derivatives bind tightly to alcohol dehydrogenase in the presence of NAD⁺ but much less tightly than the corresponding 4-substituted pyrazoles.

The 3-substituted pyrazoles could bind more poorly because the substituent binds in a sterically unfavorable mode when the pyrazole ring is bound in the same position as in the unsubstituted molecule or because the substituent prevents binding of the pyrazole ring in the usual position. The results discussed below support the first possibility.

Table I. Physical Properties of Pyrazole Derivatives

no.	3 substit	method	yield, %	mp, °C	formula ^a
1	HOCH ₂	A ^b	16	bp 135-137 ^e	C ₄ H ₆ N ₂ O ^c
2	HOCH ₂ CH ₂	A	14	bp 120-122 ^f	C ₅ H ₈ N ₂ O
3	HOCH ₂ CH ₂ CH ₂	A	15	bp 145-146 ^e	C ₆ H ₁₀ N ₂ O
4	ClCH ₂	B ^b	62	156-157	C ₄ H ₆ N ₂ Cl ₂
5	ClCH ₂ CH ₂	B	88	122-124	C ₅ H ₈ N ₂ Cl ₂
6	ClCH ₂ CH ₂ CH ₂	B	62	74-75	C ₆ H ₁₀ N ₂ Cl ₂
7	CH ₃ CO	d	64	96-97	C ₅ H ₆ N ₂ O

^a Analyses for C, H, and N were within 0.4%. ^b Prepared in ref 14. ^c N: calcd, 28.56; found, 26.08. ^d See text. ^e 0.5 mm. ^f 0.2 mm.

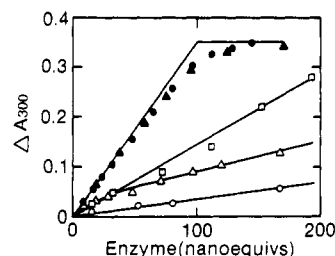


Figure 1. Spectrophotometric titration of pyrazole derivatives with horse liver alcohol dehydrogenase. In one sector (0.438-mm path length) of a double-sector cuvette, 1 mL of 100 μ M pyrazole derivative was titrated in the presence of 1 mM NAD⁺ by the addition of concentrated (40 mg/mL) enzyme, and the absorbance was recorded against a reference cuvette containing enzyme and NAD⁺ and pyrazole in separate sectors. The absorbances have been corrected for dilution. The buffer was 0.1 M sodium phosphate, pH 7.0, at 25 °C: pyrazole (●), 4-(2-hydroxyethyl)pyrazole (▲), 3-methylpyrazole (□), 3-(2-hydroxyethyl)pyrazole (△), and no pyrazole (○), which reflects binding of NAD⁺ to enzyme.¹⁵ By titrating with enzyme, contamination of a 3-substituted derivative by a 4-substituted derivative is revealed by a high slope initially due to the 4-substituted derivative and a low slope later due to the 3-substituted derivative. This method also yields dissociation constants for the predominant 3-substituted derivative, since any 4-substituted derivative would give little absorbance at 300 nm. The 3-methylpyrazole is prepared by an unambiguous route and is not contaminated by 4-methylpyrazole.

All of these 3-pyrazole derivatives inhibited the enzymatic activity competitively against ethanol and presumably bind at the substrate binding site. The 3-substituted derivatives also formed complexes with about the same absorbance maximum (295 nm) and extinction coefficient ($\Delta\epsilon_{300} = 6.0 \pm 2.5 \text{ mM}^{-1} \text{ cm}^{-1}$ for four derivatives) as the 4-substituted derivatives ($\Delta\epsilon_{300} = 7.5 \pm 2.1 \text{ mM}^{-1} \text{ cm}^{-1}$ for five derivatives). This can be demonstrated, for instance, by double difference spectroscopy in which the sample cuvette contains 85 μ N enzyme, 1 mM NAD⁺, and 0.85 mM 3-methylpyrazole and the reference cuvette contains enzyme and NAD⁺ and 3-methylpyrazole in separate compartments. By contrast, in a similar experiment with 0.1 M isoxazole the complex did not absorb significantly at 295 nm. Since the dissociation (inhibition) constants for pyrazole analogues lacking adjacent nitrogens are large [e.g., isoxazole, 24 mM; isothiazole, 8.8 mM (W.-S. Chen, unpublished results); imidazole, 7.6 mM³], it appears that both nitrogens are critical for tight binding. Comparison of dissociation constants leads to the estimate of 36 000-fold for the influence of the adjacent nitrogen on binding. These results suggest that pyrazole and the 3-substituted pyrazoles form the same chemical species when they react with the enzyme-NAD⁺ complex.

Inspection of models suggests that the poorer binding of the 3-substituted derivatives as compared to 4-substituted derivatives is due to differences in the interactions of the first two carbon atoms of the substituents with the enzyme; the third atoms could occupy similar positions.

Table II. Binding of Pyrazole Compounds to Liver Alcohol Dehydrogenase^a

substituent	dissoc constant, μ M	
	3 position	4 position
H	0.2 ^b	0.2 ^b
CH ₃	125 ^b	0.013 ^c
HOCH ₂	150 \pm 130 (3) ^d	3.6 \pm 0.5
ClCH ₂		2.7 \pm 0.1
HOCH ₂ CH ₂	170 \pm 110 (3) ^d	0.77 \pm 0.07 ^e
ClCH ₂ CH ₂	200 \pm 140 ^d	
HOCH ₂ CH ₂ CH ₂	60 \pm 40 ^d	0.56 \pm 0.05 ^e
ClCH ₂ CH ₂ CH ₂	40 \pm 30 ^d	0.02 \pm 0.001 ^e
CH ₃ CO	5100 \pm 400	
HOOC ^f	6500 \pm 600	

^a Most constants were determined by inhibition against ethanol binding. The standard errors for these constants are measures of the precision of the data or the fit of the data to the equation for competitive inhibition. ^b Determined kinetically in ref 3. Our titration results gave a value of 130 \pm 70 (3) for 3-methylpyrazole. ^c From ref 4. ^d Determined by spectrophotometric titration of enzyme with the pyrazole or by titration of the pyrazole with enzyme as in Figure 1. Standard deviations obtained for three determinations or estimated accuracies for one determination are recorded. ^e The pyrazole compound was a gift from Eli Lilly Co. ^f From ref 14.

Introduction of bulkier substituents such as acetyl or carboxyl into the 3 position of the ring seem to be especially unfavorable. Since the binding site proposed for substrates and substrate analogues is lined with hydrophobic amino acid residues,^{12,13} it might be expected that lengthening the alkyl chain in the 3 position would increase binding, and such an effect is suggested by comparison of the ethyl and propyl derivatives. Comparison of the dissociation constants for 3-methyl-, 3-(hydroxymethyl)-, and 3-(2-hydroxyethyl)pyrazoles reveals no such trend, probably because increasing hydrophobic interactions are offset by increasing steric interference.

The reasonably good affinity of the 3-substituted derivatives justified testing some of them as active-site-directed reagents. The data in Table III show that 4 is extremely reactive with 4-(*p*-nitrobenzyl)pyridine, being at least 25-times more reactive than methyl iodide. 4 at concentrations of 10 to 200 mM also very rapidly (less than 5 min) inactivated the enzyme. The extent of inactivation ranged from 36% with 10 mM reagent to 98% with 200 mM. The coenzyme NAD⁺ (1 mM) protected against inactivation, however, indicating that formation of the enzyme-NAD⁺-pyrazole complex retarded rather than facilitated the reaction. When alcohol dehydrogenase was treated with 10 mM reagent for 20 min (until the rapid inactivation had occurred), freed of excess reagent by Sephadex gel filtration, hydrolyzed in 6 M HCl for 22 and 46 h (at 110 °C), and analyzed for its amino acid composition on a Beckman 120 C analyzer, only 6 of the 26 cysteines and 13 of the 16 methionines per molecule were recovered unmodified. These results indicate that 4 extensively and nonspecifically modifies the protein and is

Table III. Reactivity of Pyrazole Derivatives

no.	pyrazole deriv	concn, mM	fract of control act. ^a		NBP react. ^b
			no addit of NAD ⁺	+ 1 mM NAD ⁺	
4	3-ClCH ₂	10	0.2	0.6	≥3000
	4-ClCH ₂ ^c	10	0.37	0.81	4
2	3-HOCH ₂ CH ₂	100	0.53	0.11	
5	3-ClCH ₂ CH ₂	10	1.0	0.09	0.8
		1	0.9	0.14	
		0.1	0.95	0.48	
6	3-ClCH ₂ CH ₂ CH ₂	10	0.78	0.1	0.2
	4-ClCH ₂ CH ₂ CH ₂	10	0.52	0.04	

^a The activity obtained when 10 μ L of the reaction mixture containing the stated concentration of reagent and NAD⁺ was diluted into 1 mL of the enzyme assay mixture,¹⁶ and the initial velocity of NADH production was measured. The enzyme (1 mg/mL) was incubated with the reagent for less than 5 min in 0.1 M Na₂P₂O₇, adjusted to pH 8.0 with H₃PO₄ at 25 °C. ^b Relative chemical reactivities with 4-(*p*-nitrobenzyl)pyridine were determined by the method of Baker and Jordaan.¹⁷ The reactivities are expressed as the rate of change of absorbance at 570 nm divided by the final concentration of the alkylator in the reaction mixture (at 37 °C). The reactivities of model compounds for comparison were: bromoethanol, 0.3; bromoacetic acid, 22; methyl iodide, 45.

^c Prepared in ref 14.

not a useful active-site-directed reagent. Reaction of 4 with free cysteine at neutral pH yielded a new compound that chromatographed near isoleucine on the amino acid analyzer. This compound was not altered by hydrolysis in 6 M HCl for 24 h at 110 °C. The reagent also modified free methionine, but the product, presumed to be the cationic pyrazolymethylsulfonium salt, did not appear on the amino acid analyzer.

4-(Chloromethyl)pyrazole was also quite chemically reactive and appeared to inactivate the enzyme. NAD⁺ (1 mM), NADH (0.2 mM), and AMP (2 mM) all protected against inactivation, indicating again that the reaction was nonspecific.

The other (chloroalkyl)pyrazoles (and 2) were not very reactive with 4-(*p*-nitrobenzyl)pyridine, but they appeared to cause rapid inactivation of the enzyme in the presence, but not in the absence, of NAD⁺ (Table III). This is probably due to reversible inhibition caused by formation of a tight enzyme-NAD⁺-pyrazole complex. The rate of formation of this complex was too fast to measure with conventional mixing techniques, but stopped-flow studies give a rate of $3 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ with pyrazole itself.¹⁸ An estimate of the rate of dissociation of 5 and 6 from the ternary complexes was obtained by diluting a solution containing 0.94 μ N enzyme, 1 mM NAD⁺, and 1 mM 5 or 6 100-fold into the usual assay mixture,¹⁶ which contained 0.55 M ethanol and 1.75 mM NAD⁺ at pH 9 and 25 °C, and measuring the rate of increase of absorbance at 340 nm due to NAD⁺ reduction catalyzed by uninhibited enzyme. (The high concentration of ethanol should largely prevent reassociation of pyrazole.) The rate of NAD⁺ reduction (measured as the tangents to the slopes at various times) increased from the initial value of about 10% of the original activity up to 48–58% of the original activity at 40 min, with an apparent first-order rate constant of $1 \times 10^{-3} \text{ s}^{-1}$. If the enzyme was not preincubated with NAD⁺ and the pyrazole compound but was instead diluted into the assay mixture in which there was 0.01 mM (chloroalkyl)pyrazole, the rate of NAD⁺ reduction decreased in 20–40 min from 100 to 70–80% of the original activity, measured in the absence of the pyrazoles. Thus,

the steady-state rates of NAD⁺ reduction were similar whether or not the enzyme was preincubated with NAD⁺ and (chloroalkyl)pyrazole. The lag phases observed in assays with pyrazole itself have been described previously.³ Our experiments demonstrate that 5 and 6 inhibit reversibly but that the rate of dissociation of the inhibited complex is slow, possibly due to slow decomposition of the adduct in Scheme I.

Furthermore, the ternary enzyme-NAD⁺-(chloroalkyl)pyrazole (5 or 6) complex did not appear to undergo a slow irreversible reaction to form inactive enzyme. Incubation for 5 min, 2 h, or 18 h of enzyme with 1 mM NAD⁺ and 1 mM 5 or 6 in 33 mM sodium phosphate buffer at pH 7 and 25 °C, which reduced activity to about 20%, and removal of reagents by gel filtration on Sephadex G-50 gave enzyme with the same ($67 \pm 7\%$ of the original) activity.

Thus, the 3-substituted pyrazole derivatives studied in this work do not have the characteristics required for specific inactivation of the enzyme. The lack of reactivity may be due to the positioning of the reactive substituent (in the enzyme-NAD⁺-pyrazole complex) in the hydrophobic region away from the reactive sulfurs. Alternatively, the 3 substituent may not be able to bind near the sulfurs because of steric interference. Lack of reactivity of 5 and 6 could be due to low inherent chemical reactivity, but 4 certainly is reactive enough. These studies lead us to suggest that effective reagents should have a large hydrophobic group in the 4 position to facilitate binding and to properly orient a small reactive substituent in the 3 position.

Experimental Section

Infrared spectra were recorded on KBr pellets with a Perkin-Elmer 21 instrument. NMR spectra were obtained at 60 MHz on a Varian A60 in Me₂SO-*d*₆ with tetramethylsilane as internal standard. Elementary analyses for C, H, and N were carried out by Galbraith Laboratories and The University of Iowa, Department of Chemistry. Melting points are uncorrected.

3-(Hydroxymethyl)pyrazole (1). Method A. To 6 g (0.14 mol) of diazomethane in 1 L of anhydrous diethyl ether was added 8.0 g (0.14 mol) of freshly distilled propargyl alcohol. The mixture was stirred gently for 1 week at room temperature, at which time the ether was removed at reduced pressure. The fraction boiling at 135–137 °C at 0.5 mm was collected. Most of the unreacted propargyl alcohol could be recovered.

3-(2-Chloroethyl)pyrazole Hydrochloride (5). Method B. To 0.5 g (4.5 mmol) of (hydroxyethyl)pyrazole was added 25 mL of concentrated HCl. The solution was evaporated to dryness, taken up in absolute ethanol, and reevaporated. The crude salt was added to 10 mL of thionyl chloride, and the mixture was heated under reflux for 30 min. The thionyl chloride was removed at reduced pressure, and the crude product was taken up in 25 mL of concentrated HCl and evaporated. The white crystalline product was washed with 5 mL of cold acetone, triturated with ether, and dried in vacuo to yield 0.67 g (88%) of the (chloroethyl)pyrazole hydrochloride salt.

3-Acetylpyrazole (7). To 2.3 g of diazomethane (0.055 mol) in 500 mL of anhydrous diethyl ether was added dropwise 2.5 g (0.035 mol) of butynone and the mixture was stirred gently at room temperature. The yellow-green color of diazomethane disappeared overnight, and the ether was removed at reduced pressure. A white crystalline product formed and was washed with 50 mL of warm hexane and dried in vacuo to afford 2.5 g of the product: IR (Nujol) 1680 cm⁻¹ (CO); NMR (CD₃Cl) δ 2.6 (s, CH₃), 6.9 (d, ring CH), 7.8 (d, ring CH).

Enzymology. The EE isoenzyme was purified¹⁹ and freed of ethanol by dialysis and gel filtration so that less than 1 equiv of NADH was produced per subunit of enzyme (at 1 mg/mL) in the presence of 1 mM NAD⁺ at pH 8 and 25 °C over 3 h. Inhibition constants for pyrazoles were determined by competition against varied concentrations of ethanol (0.4–2 mM) at a fixed concentration of NAD⁺ (1 mM) in 33 mM sodium phosphate buffer,

pH 7, at 25 °C using 10 nN enzyme. A Cary 118 C spectrophotometer with a 0.05-A scale was used to record the assays. Steady-state velocities (i.e., activity between 3 and 4 min of reaction) were used, since the extent of inhibition is time dependent. The data were fitted to the equation for competitive inhibition using Cleland's program,²⁰ which computes the standard errors.

The inhibition constants obtained for the new 3-substituted pyrazoles were about tenfold higher than the values for the corresponding 4-substituted pyrazoles, but the 3-substituted derivatives may have been slightly contaminated by the 4-substituted derivatives. Therefore, dissociation constants for the 3-substituted derivatives were determined by difference titration, with the pyrazole compound, of 19 to 27 μ N enzyme in the presence of 1 mM NAD⁺ in 0.1 M sodium phosphate buffer, pH 7.0, at 25 °C, in one sector (0.438-mm path length) of a double-sector cuvette against a reference cuvette containing enzyme in one sector and NAD⁺ and pyrazole in the other sector. Alternatively, or in addition, the pyrazole was titrated with enzyme as in Figure 1. On the basis of an extinction coefficient of 7200 M⁻¹ cm⁻¹ at 300 nm, the concentration of enzyme-NAD⁺-pyrazole complex was calculated, after subtracting 0.25 A₃₂₈ from the A₃₀₀ as a correction for the formation of small amounts of enzyme-NADH complex.¹⁵ The dissociation constant was computed by a linear least-squares fit to the data graphed as a Scatchard plot. Titrations with 3-methylpyrazole and 4-substituted pyrazoles with dissociation constants larger than 1 μ M gave dissociation constants approximately the same as the inhibition constants for these compounds.

Irreversible inactivation of 1 mg of enzyme/mL of 0.1 M Na₂P₂O₇ buffer adjusted to pH 8 with H₃PO₄ was determined at 25 °C with reagent concentrations ranging from 1 to 100 mM by assaying 10 μ L of reaction mixture in 1 mL of a standard assay mixture¹⁶ after various times of reaction, usually over a 24-h period. The effect of 2 mM AMP or 1 mM NAD⁺ or 0.2 mM NADH was routinely studied in order to be able to identify reagents that reacted specifically in the presence of NAD⁺. In order to determine if the reagent affected the activity of the enzyme in the assay, a control was run. To the assay mixture containing NAD⁺ and ethanol was added the same amount of reagent as would be present when the reaction mixture was diluted (100-fold) into the assay mixture. After the assay mixture was stirred, unmodified enzyme in pH 8 buffer was added and the steady-state rate was determined. If the reagent did not strongly inhibit the enzyme, the activity in the control should be the same as the assay of the unmodified enzyme, but, as discussed above, several of the

reagents did strongly inhibit activity. Second-order rate constants were calculated on the assumption that the reaction was bimolecular.

Amino acid analyses were performed by the usual methods.²¹

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Quantitative Structure-Activity Relationships for Biguanides, Carbamimidates, and Bisbiguanides as Inhibitors of *Streptococcus mutans* No. 6715

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Thirty-seven compounds, including 17 biguanides, 6 carbamimidates, and 14 bisbiguanides, were evaluated for potential antiplaque activity by measuring their minimum inhibitory concentrations [MIC (M)] against *Streptococcus mutans* no. 6715. Linear regression analysis was conducted with the log 1/MIC (M) values and log P, π , σ , and MR. The best correlation for the biguanides ($r^2 = 0.92$) was obtained with log P and (log P)². When the biguanides were included with the carbamimidates, essentially the same correlation ($r^2 = 0.91$) was obtained with log P and (log P)². The best correlation for the bisbiguanides ($r^2 = 0.70$) was also obtained with log P and (log P)². Use of an indicator variable (I) for the bisbiguanides allowed all three groups to be included in one equation, which accounted for over 87% of the variance in the data for inhibition of bacterial growth. The results from the classical parabolic model were also compared with those from the recently developed bilinear model.

The organism *Streptococcus mutans* no. 6715 plays a major role in the formation of dental plaque. Dental plaque, in turn, is the primary cause of caries and per-

iodontal disease.¹ One approach for the prevention of these diseases has involved the use of antibacterial agents. Chlorhexidine and its analogues have been shown to be