Belgian National Fund for Scientific Research. We thank Dominique Brabants for her dedicated editorial assistance and Anita Van Lierde, Frieda De Meyer and Anita Camps for their efficient technical assistance.

Registry No. 1, 69075-43-0; **3a**, 133040-26-3; **3b**, 133040-27-4; **3c**, 133040-28-5; **3d**, 133040-29-6; **3e**, 133070-82-3; **4a**, 133070-83-4; **4b**, 133040-30-9; **4c**, 133040-31-0; **4d**, 133040-32-1; **4e**, 133040-33-2; **6a**, 698-16-8; **6b**, 28123-63-9; **7**, 74213-24-4; **8** (R = CH_3), 79-24-3; **8** (R = C_2H_5), 25322-01-4; **9**, 103-71-9; **10**, 133040-34-3; **11**, 133040-35-4; **12a**, 107-20-0; **12b**, 70-11-1; **12c**, 78-95-5; **13a**, 133040-40-1; **13b**, 133040-41-2; **13c**, 133040-42-3; **14a**, 133040-36-5; **14b**, 133040-37-6; **14c**, 133040-38-7; **15**, 73446-40-9; **16**, 696-59-3; **17**, 133040-39-8.

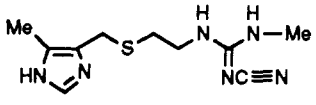
Substituent Effect on the Stereochemistry of H_2-Receptor Antagonists of the Phenylformamidine Series. A Conformation-Dependent Mode of Interaction with the H_2 Receptor

Arturo Donetti,*,† Harold M. M. Bastiaans,‡ Klaas Kramer,‡ Giuseppe Bietti,† Enzo Cereda,† Enrica Dubini,† Maurizio Mondoni,† Aalt Bast,‡ and Hendrik Timmerman‡

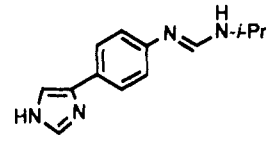
Research Laboratories of Istituto De Angeli, Via Serio, 15, I-20139 Milan, Italy, and Department of Pharmacochemistry, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands. *Received September 13, 1990*

The influence of alkyl substitution on the stereoisomerism of the formamidine cation (*E,E* vs *E,Z*) of several N-substituted (imidazolylphenyl)formamidines (**1–10**) was investigated. As (imidazolylphenyl)formamidines having alkyl substituents of more than three carbon atoms bind to H_2-receptor preparations in a pseudoirreversible mode causing unsurmountable antagonism, the four isomeric butylformamidines (**5–7** and **9**) having comparable lipophilic character but different *E,E*/*E,Z* composition were investigated in H_2-receptor assays to determine quantitatively any difference in their pseudoirreversible inhibitory pattern. It was found that the geometry of the formamidine cation is affected by the steric bulk of the substituent on the formamidine nitrogen. A relationship between the percentage of the *E,E* conformation of the formamidine cation and degree of pseudoirreversible antagonism was also found. The present studies support the hypothesis that bidentate hydrogen bonding plays an important role in the interaction of (imidazolylphenyl)formamidines with the H_2 receptor.

The search for H_2 antagonists by the structural modification of cimetidine has led to the discovery of a number



cimetidine



mifentidine (1)

of new compounds.[1] Chemical variations of this prototype structure have focused mainly on the two molecular determinants for H_2-receptor antagonism, i.e. the imidazole ring and the cyanoguanidine moiety.

While the imidazole ring was amenable to replacement only by a limited number of moieties (e.g. aminomethylfuran, guanidinothiazole, aminomethylbenzene), manipulation of the cyanoguanidine part has led to a wide variety of amidine-type structures which were introduced on structural parts of classical H_2-receptor antagonists.[1,2] Thiourea, cyanoguanidine, nitroethenediamine, and sulfamoylamidine are examples of acyclic amidine systems. Aminopyrimidones, -thiadiazoles, -thiatriazines, and -thiazoles (benzo- or thieno-fused) may be considered cyclized amidine correspondents. Most of them possess a reduced basicity conferred by the presence of electronegative substituents or by the inherent sp^2 character of their nitrogens' lone pairs.

Ganellin and co-workers,[3] in a study aimed at analyzing quantitatively differences in the antagonist activity associated with these moieties, have pointed out the importance of the orientation of the dipole moment associated with these amidine systems. Their findings suggest that interaction of these structural parts with the H_2 receptor might be largely determined by their hydrogen-bonding ability. Recent studies[2,4] on mifentidine (**1**),[5] a new type of H_2-receptor antagonist having a phenylformamidine structure, have led us to suggest a general model for the interaction of amidino groups with the H_2 receptor. The model proposes that these moieties bind to a common binding site in two possible modes, as depicted in Figure 1.

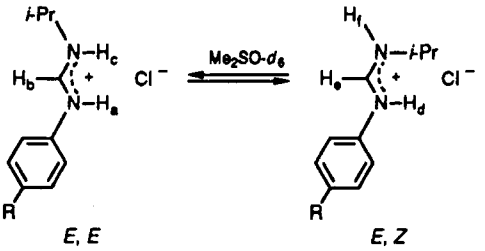
It is implicit from this model that in order to establish an effective bidentate coupling, a syn relationship of the two hydrogens on the amidine nitrogens is required. The model also implies that the formation of the cyclic complex is energetically compatible and that the amidine group is present at the receptor in a proper alignment. Provided the latter condition is fulfilled, whether the formamidine binds in its protonated form to an ionized acidic moiety, as in Figure 1 (part a), or in its neutral form to an unionized acidic counterpart (part b), the energy of binding will be influenced by its steric bias to assume the required syn relationship of NH bonds (i.e. the *E,E* configuration

* Address for correspondence: Dr. Arturo Donetti, Dept. of Medicinal Chemistry, Istituto De Angeli, Via Serio, 15, I-20139 Milan, Italy.
† Istituto De Angeli.
‡ Vrije Universiteit Amsterdam.

(1) For a recent review on the medicinal chemistry of H_2-receptor antagonists see: Donetti, A. In *Actualité de Chimie Therapeutique*; Chatenay-Malabry Cédex, 1988; pp 227–246.
(2) Donetti, A.; Cereda, E.; Ezhaya, A.; Micheletti, R. *J. Med. Chem.* **1989**, *32*, 957–961.
(3) Young, R. C.; Durant, G. J.; Emmet, J. C.; Ganellin, C. R.; Graham, M. J.; Mitchell, R. C.; Prain, H. D.; Roantree, M. L. *J. Med. Chem.* **1986**, *29*, 44–49.
(4) Bazzano, C.; Vanoni, P. C.; Mondoni, M.; Gallazzi, A.; Cereda, E.; Donetti, A. *Eur. J. Med. Chem.* **1986**, *21*, 27–33.
(5) Donetti, A.; Cereda, E.; Bellora, E.; Gallazzi, A.; Bazzano, C.; Vanoni, P. C.; Del Soldato, P.; Micheletti, R.; Pagani, F.; Giachetti, A. *J. Med. Chem.* **1984**, *27*, 380–386.

0022-2623/91/1834-1772$02.50/0

Table I. ^1H NMR Data Relative to the Formamidinium Protons of Mifentidine (1) and its Pyrazole Analogue (2)



compd	R	H	δ^a	J, Hz	H	δ^a	J, Hz	% Isomeric Species E,E/E,Z
1		a	12.10 (br)	a,b, nd	d	12.10 (br)	d,e, nd	34.6/65.4
		b	9.03 (d)	b,c, 11.5	e	8.55 (d)	e,f, 6.1	
		c	10.36 (m)		f	10.72 (m)		
2		a	11.89 (d)	a,b, 13.4	d	11.89 (d)	d,e, 13.4	41.2/58.8
		b	9.14 (m)	b,c, 13.0	e	8.55 (m)	e,f, 6.5	
		c	10.35 (m)		f	10.81 (m)		

[a] br = broad signal; d = doublet; m = multiplet; nd = not detectable.

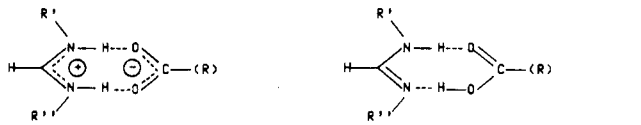


Figure 1. Possible interaction modes of amidine moieties with an acidic site of the H_2 receptor: (R) = receptor; R′ and R″ = amidine substituents.

of the amidine system, Figure 2) prior to or as a result of the interaction process.

The object of this work was to assess the validity of our interaction model in the class of (imidazolylphenyl)formamidines. Therefore, NMR studies were performed to rationalize the influence of substituents on the relative configuration of the amidine cation (*E,E* vs *E,Z*). Since (imidazolylphenyl)formamidines having alkyl substituents of more than three carbon atoms bind to H_2-receptor preparations in a pseudoirreversible mode causing unsurmountable antagonism,[6] the four isomeric butylformamidines having comparable lipophilic character but different *E,E*/*E,Z* composition were investigated in H_2-receptor assays to determine quantitatively the differences in their inhibitory pattern. According to our model, a high *E,E*/*E,Z* ratio of amidinium forms would reflect a high steric bias for effective hydrogen bonding. If a positive correlation between this isomeric ratio and the degree of unsurmountable antagonism is found, this would support the hypothesis that bidentate binding controls interaction at the receptor site in this class of H_2-receptor antagonists.

Results and Discussion

The Stereochemistry of the Formamidine Cation. Protonation of N,N′-disubstituted formamidines occurs at the imino nitrogen, giving a conjugate acid which can exist in four theoretical planar forms (Figure 2). Nuclear magnetic resonance spectra of mifentidine (1) in DMSO-d_6 give two distinguishable sets of signals for the protons H_a–H_f, which were assigned on the basis of multiplicity, decoupling experiments, and the general observation that an N*H* aryl is downfield of an N*H* alkyl. Table I reports the ^1H NMR data relative to the three protons of the two forms of the formamidinium cation of mifentidine. In Me_2SO-d_6 mifentidine (1), as the dihydrochloride, is

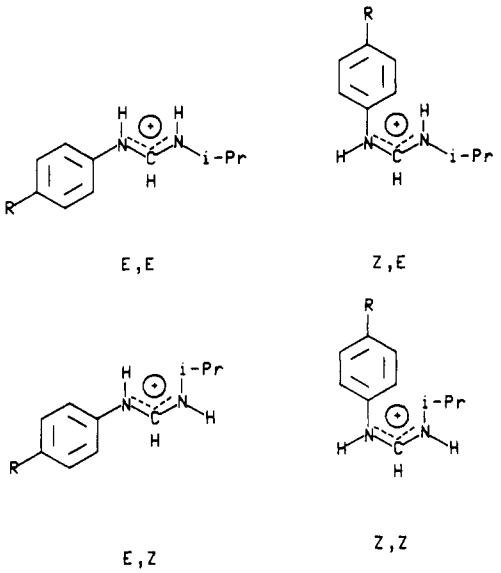
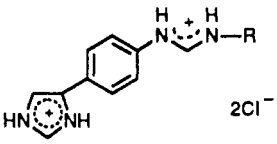


Figure 2. Amidinium monocation of mifentidine (R = 4-imidazolyl) and its possible planar isomeric forms.

present as a mixture of two geometric forms with different relative abundances, as calculated by the appropriate integral values. The more abundant species shows coupling constants ($J_{e,f}$ = 6.1 Hz) in accord with a cis geometry (*Z*), while the less abundant has a correspondent $J_{b,c}$ = 11.5 Hz, which indicates a transoid geometry (*E*). Unfortunately, H_a and H_d show broadened doublets, and their coupling constants ($J_{a,b}$ and $J_{d,e}$) are undetectable. However, mifentidine analogue **2**, in which the imidazole is replaced by a pyrazole ring, displays sharp H_a and H_d doublets, which allows determination of their coupling constants. Compound **2** presents and *E,Z* geometry ($J_{d,e}$ = 13.4 Hz, $J_{e,f}$ = 6.5 Hz) for the more abundant species, and an E,E geometry ($J_{a,b}$ = 13.4 Hz, $J_{b,c}$ = 13.0 Hz) for the less abundant one, which allows a plausible assignment to the geometry of the corresponding imidazole analogue mifentidine (1). The geometry of the mifentidine derivatives in which the isopropyl group is replaced by other alkyl moieties (**3**–**10**) was determined similarly. Integration of the C*H* formamidine protons following D_2O exchange provides a facile measure of the equilibrium concentrations at 20 °C (Table II) of the slowly interconvertible *E,Z* and *E,E* forms present in mifentidine (1) and its derivatives (**3**–**10**).

(6) Kramer, K.; Bast, A.; Timmerman, H. *Agents Actions* **1987**, *21*, 41–48.

Table II. Compounds, pA_2 Values, Substituent Parameters, and Data Used in the Formulation of Equations 1 and 2



compd	R	pA_2 (atrium)	slope (±SE)	E'_s	B_1	% E,E/E,Z	log $C_{E,E}$
1	*i*-Pr	7.55 ± 0.27	1.17 (0.05)[a]	−0.48	1.90	34.6/65.4	1.54
3	Me	5.92 ● 0.167	1.08 (0.04)[b]	0.00	1.52	22.5/77.5	1.35
4	Et	7.37 ± 0.163	0.67 (0.06)[a]	−0.08	1.52	28.5/71.5	1.45
5	*n*-Bu	7.95 ± 0.14	1.35 (0.10)[b]	−0.31	1.52	26.2/73.8	1.42
6	*i*-Bu	8.28 ● 0.14	1.75 (0.12)[a]	−0.93	1.52	27.2/72.8	1.43
7	*s*-Bu	7.85 ± 0.05	0.92 (0.23)[b]	−1.00	1.90	36.4/63.6	1.56
8	Et_2CH	7.50 ± 0.103	1.54 (0.26)[b]	−2.00	2.13	41.5/58.5	1.62
9	*t*-Bu	7.95 ± 0.09	1.07 (0.06)[a]	−1.43	2.60	100/0.0	2.00
10	$EtMe_2C$	8.12 ● 0.23	1.02 (0.25)[b]	−2.28	2.60	100/0.0	2.00

[a] From ref 5. [b] Results obtained in a series of separate experiments using the same procedure described in ref 5.

Substituent Effect on Formamidine Stereoisomerism. The nature of the alkyl substituent on the formamidine nitrogen clearly affects the geometry of the formamidinium cation. It was soon apparent that this effect was ascribable to the shape or steric bulk of the substituent itself, since small or linear groups (e.g. methyl or *n*-butyl) stabilize preferentially the *E,Z* configuration, whereas highly branched alkyl groups (e.g. *tert*-butyl) stabilize the *E,E* form (Table II). In order to find a physical-chemical property capable of describing such an effect, a steric descriptor was sought which could be examined in a correlation analysis. Only very poor correlations were obtained with use of molar refractivity (MR)[7] or molecular weight (MW), molecular connectivity indexes (χ and related variants),[8] the molecular shape and bulk descriptors (V^a and G),[9] and Austel's substituent branching parameter (S_b).[10]

Satisfactory equations were instead obtained when the percent concentration value of the *E,E* form, expressed as log $C_{E,E}$, was fitted with the revised Taft steric constant E'_s (eq 1),[11] or better, with the Verloop's Sterimol parameter B_1 (eq 2) (Table II).[12]

$$\log C_{E,E} = -0.24\ (\pm 0.17)E'_s + 1.36\ (\pm 0.20) \qquad (1)$$

$$n = 9,\ r = 0.79,\ s = 0.16,\ F_{1,7} = 11.8$$

$$\log C_{E,E} = 0.53\ (\pm 0.11)B_1 + 0.58\ (\pm 0.21) \qquad (2)$$

$$n = 9,\ r = 0.97,\ s = 0.06,\ F_{1,7} = 137.6$$

Here, n is the number of compounds, r is the correlation coefficient, s is the standard deviation, F is the Fisher's F test value, and the values in parentheses are the 95% confidence limits of the regression coeffficients.

The above equations suggest that the steric effect of the substituents on the relative concentrations of the two isomeric forms is related to the volume of the substituent portion close to the attachment atom of the formamidine system. Both B_1 and E'_s essentially measure the minimum widths of substituents, and the observed dependence of log $C_{E,E}$ on these parameters reflects the tendency of substituents to change their conformation in a way that minimizes steric interaction. Highly hindered alkyl groups will push the amidinium system to assume the *E,E* configuration so as to relieve steric constraint.

Pharmacology and Conformation-Activity Relationship. Recently, we performed receptor [^{3}H]tiotidine displacement-binding studies[6] with some mifentidine analogues bearing alkyl groups larger than isopropyl, i.e. $C(CH_3)_3$, $C(CH_3)_2CH_2CH_3$, and $CH_2C(CH_3)_3$. Results indicated that these compounds bind irreversibly and cause unsurmountable H_2 antagonism via negative cooperativity.

The irreversible shift of the histamine dose-response curve by the mifentidine analogues was not always paralleled by a depression of the curve. Moreover, the analogues did not give a decrease in the number of [^{3}H]tiotidine-binding sites. Therefore, we performed functional studies to quantify the histamine dose-response shift rather than radioligand-binding experiments in order to investigate the proposed relationship between the irreversible antagonism and the stereochemical configuration of the butylformamidines (**5–7** and **9**).

The compounds were tested for their H_2 antagonism on the right atrium of the guinea pig. Reported in Table II are the pA_2 values of a series of alkylphenylformamidines along with pertinent physicochemical parameters. Addition of an H_2 antagonist to the right atrium will cause a shift to the right of the histamine dose-response curve (curves 2 in Figure 3a–d). In the case of reversible antagonism the dose-response curve, recorded after washing out of the antagonist, will completely return to the original histamine dose-response curve (curves 1 in Figure 3a–d). If an irreversible H_2 antagonist is used, the histamine dose-response curve will not return to its control situation. The histamine dose-response curves constructed after washing out the butylformamidines under study (**5–7** and **9**) shifted back to the left, but a complete recovery of the original histamine dose-response curve was not found (curves 3 in Figure 3a–d). The degree of irreversible binding equals the relative percent recovery of the histamine dose-response curve after washing out the antagonist, and this can be calculated by eq 3,[6] where EC_{50} is the

$$\%\ \text{recovery} = \left(1 - \frac{EC_{50}^{B*} - EC_{50}}{EC_{50}^{B} - EC_{50}}\right) \times 100 \qquad (3)$$

concentration of agonist at 50% maximal effect EC_{50}^B is the concentration of agonist at 50% maximal effect when antagonist (10^{-7}) is present, and EC_{50}^{B*} is the concentra-

(7) Dunn, W. J., III *Eur. J. Med. Chem.* **1977**, *12*, 109–112.
(8) Kier, L. B.; Hall, L. H. *Molecular Connectivity in Chemistry and Drug Research. Medicinal Chemistry*; Academic Press, Inc.: New York, 1976.
(9) Meyer, A. Y. *J. Chem. Soc. Perkin Trans. 2* **1986**, 1567–1572.
(10) Austel, V.; Kutter, E.; Kalbfleisch, W. *Arzneim-Forsch.* **1979**, *29*, 585–587.
(11) MacPhee, J. A.; Panaye, A.; Dubois, J.-E. *Tetrahedron* **1978**, *34*, 3553–3562.
(12) Verloop, A.; Hoogenstraaten, W.; Tipker, J. In *Drug Design*; Academic Press Inc.: New York, 1976; Chapter 4, Vol. VII, pp 165–207.

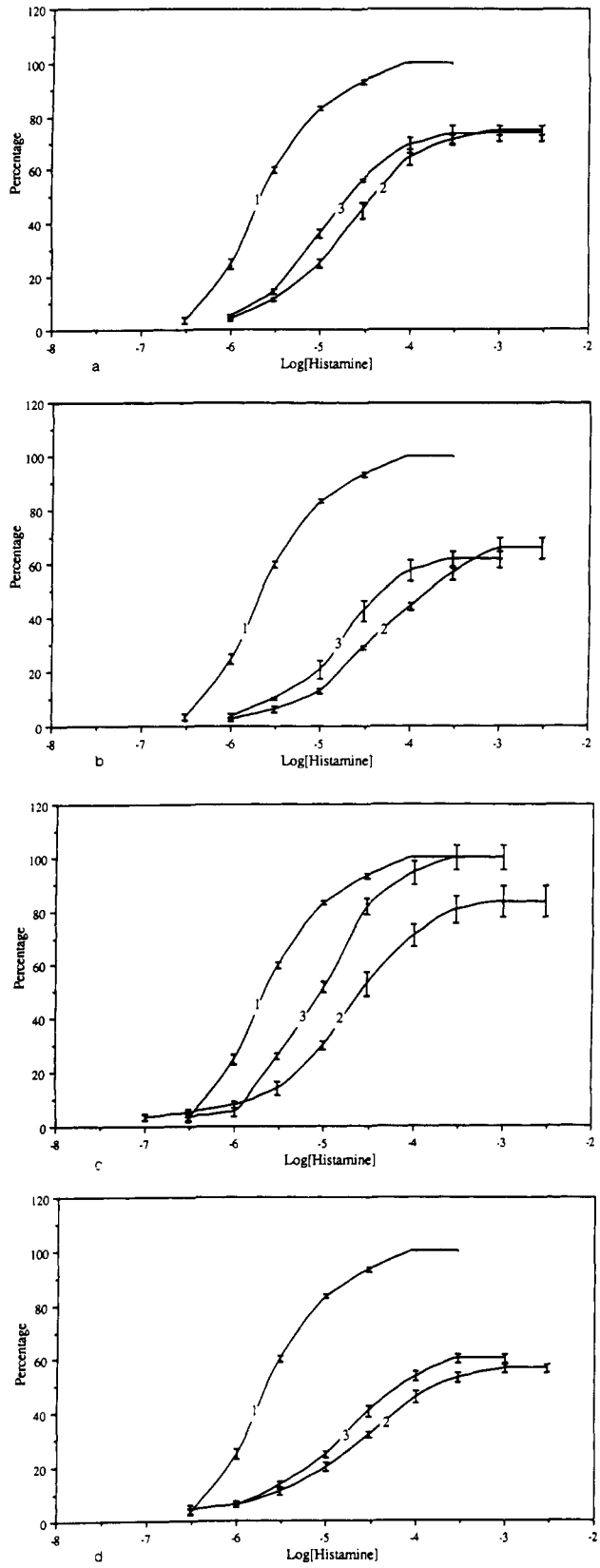


Figure 3. Dose–response curves of histamine alone (1), of histamine in the presence of the antagonist (2), and of histamine after a wash-out period of 45 min (3). a–d Show the effects of the four antagonists **5–7**, and **9**, respectively. Each point represents the mean of five independent experiments; *y* bars indicate the SEM. All antagonists were tested at $C = 10^{-7}$.

tion of agonist at 50% maximal effect after the washing procedure.

The results reported in Table III are the mean of five independent experiments.

Table III. Percent Recovery of the Washing-Out Procedures of the Four Isomeric Butylformamidines

compd[a]	R	% recovery[b]	% *E,E*[c]
5	*n*-Bu	62	26.2
6	*i*-Bu	67	27.2
7	*s*-Bu	60	36.4
9	*t*-Bu	34	100.0

[a] All compounds were tested as their dihydrochloride salts. [b] Calculated with eq 3. [c] See Table II.

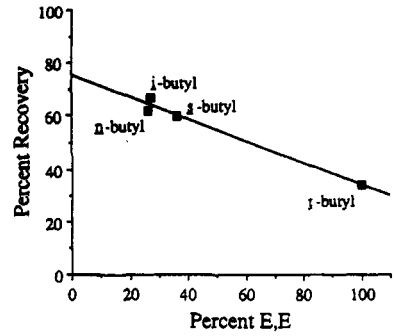


Figure 4. Percent recovery of cumulative histamine dose–response curve calculated according to eq 3, plotted against the percent of *E,E* isomer of the butylformamidines reported in Table III.

In this equation (3) the reversible or irreversible nature of antagonist emerges in a quantitative way. Completely reversible antagonists will show 100% recovery and, in an absolute sense, irreversibility will lead to 0% recovery. In eq 3 the potency of an antagonist is taken into account as well.

The four isomeric butylformamidines **5–7**, and **9** proved to be very potent H_2-receptor antagonists (Table II). After the washing-out procedure, only partial recovery of the histamine dose–response curve was observed (Figure 3a–d). As seen from Figure 4, there was a relationship between the percent of the *E,E* form measured for the four butyl derivatives and the percent recovery of the histamine dose–response curve. Clearly, the number of data points used for this relationship is rather limited due to the constraint imposed by the number of possible isomers of the butyl group. On the other hand, the inclusion of other *N*-alkylformamidines of different lipophilicity would introduce an additional factor contributing to irreversible antagonism, thus obscuring the steric contribution to the binding. Even with these limitations, the present study supports the hypothesis that a strong *E,E* amidinium ion–carboxylate interaction, provided by a bidentate binding of the type described by Walker[13] (Figure 1), is responsible for the observed degree of pseudoirreversible antagonism displayed by the butyl analogues of mifentidine.

Experimental Section

Chemistry. Melting points are uncorrected and were taken on a Büchi capillary melting point apparatus. Analytical values for the new compounds here described were within ±0.4% of the theoretical values. Preparation of compounds **1–7** and **9** was described previously.[5] Compound **8** was prepared similarly by reaction of *N*-cyano-*N'*-(1*H*-imidazol-4-ylphenyl)formamidine with 3-aminopentane in water, following the procedure described in ref 5 for compound **17**. The compound was obtained as the dihydrochloride in 81.5% yield, mp 180–182 °C. Anal. ($C_{15}H_{22}Cl_2N_4$) C, H, N, Cl. Compound **10** was prepared according to the method reported in ref 5 for compound **20**, starting from 4(5)-(4-aminophenyl)-1*H*-imidazole and (1,1-dimethylpropyl)-

(13) Walker, J. *J. Chem. Soc.* **1949**, 1996.

formamide. The compound was obtained as the dihydrochloride in 52.3% yield, mp 260–262 °C. Anal. ($C_{15}H_{22}Cl_2N_4$) C, H, N, Cl.

^{1}H NMR Spectra. ^{1}H NMR spectra were recorded at 20 °C (±1) on a Varian VXR-2000 spectrometer equipped with a VXR-4000 data system. Chemical shifts are reported relative to tetramethylsilane, utilizing the central peak of Me_2SO as reference signal (2.49 ppm). Solutions were prepared by accurately weighing 40 mg of the appropriate (imidazolylphenyl)formamidine in 0.7 mL of Me_2SO-d_6. The percent concentrations of the two occurring stereoisomeric forms, *E,Z* and *E,E*, were determined after addition of 100 μL of D_2O, and integration of the decoupled singlets relative to the methine formamidine protons. Precision of NMR determinations of *E,Z*/*E,E* ratios was within ±2 of the reported values.

Pharmacology. Male guinea pigs weighing 350–400 g were killed by a blow on the head and bled. The heart was rapidly removed and, after preparation of the spontaneously beating right atrium, this atrium was mounted for isometric recording in a water-jacketed organ bath (37 °C) containing a Krebs solution of the following composition (mM): NaCl (117.5), KCl (5.6), $MgSO_4$ (1.18), $CaCl_2$ (2.5), NaH_2PO_4 (1.28), $NaHCO_3$ (25), glucose (5.5) (pH 7.4 gassed with 5% CO_2 in O_2). Preparation of the right atrium was also done in a Krebs solution of the same composition. To each preparation a passive force of 0.5 g was applied. After an equilibration time of 30 min with four intermittent washings, a cumulative dose–response curve with histamine was recorded on a Grass Model 79 Polygraph using an isomeric Grass Ft.03 transducer. A wash-out period of 30 min was followed during which the bath fluid was changed four times. After incubation of the antagonist (bath concentration 10^{-7}) for 30 min, a dose–response curve of histamine was recorded in the same bath and a second wash-out period was followed for 45 min during which the bath fluid was changed six times. After this extensive washout a third dose–response curve was recorded. Then, the EC_{50} of histamine and the pA_2's of the H_2 antagonists were determined. After the extensive wash-out period the histamine dose–response curves did not completely return to the original histamine dose–response curve. The percent recovery of the histamine dose–response after washing out the antagonist can then be obtained by eq 3.