Registry No. 1, 76807-56-2; **2a**, 52787-14-1; **2b**, 77959-48-9; **2c**, 88392-91-0; **2d**, 88392-92-1; **3a**, 88392-93-2; **3b**, 88392-94-3; **3c**, 88392-95-4; **3d**, 88392-96-5; **4a**, 88392-97-6; **4b**, 88392-98-7; **4c**, 88392-99-8; **4d**, 88393-00-4; **5a**, 85325-88-8; **5b**, 88393-01-5; **5c**, 88393-02-6; **5d**, 88393-03-7; **6a**, 85345-35-3; **6b**, 88393-04-8; **6c**, 88393-05-9; **6d**, 88393-06-0; **7**, 6232-88-8; **8**, 88382-49-4; **9**, 88393-07-1; **10**, 84851-57-0; **11**, 88393-08-2; **12**, 59584-27-9; **13**, 88393-09-3; **14**, 88393-10-6; **15**, 88393-11-7; diethyl L-glutamate hydrochloride, 1118-89-4; benzenethiol, 108-98-5; dihydrofolate reductase, 9002-03-3; thymidylate synthase, 9031-61-2.

(Imidazolylphenyl)formamidines. A Structurally Novel Class of Potent Histamine H_2 Receptor Antagonists†

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Structure–activity considerations of N^{α}-guanylhistamine, the first compound found with detectable H_2-antagonist activity, led to the synthesis of a series of conformationally rigid guanylhistamine analogues, namely, (imidazolylphenyl)guanidines, imidazolylbenzamidines, and (imidazolylphenyl)formamidines. It was found that in the guanidine and benzamidine classes, the meta-substituted derivatives (**3**, **4**, **7**, and **8**) possessed H_2-antagonist activity, whereas in the class of formamidines, only the para-substituted derivative **10** was found active. A subsequent increase in the size of the substituent at the formamidino group of **10** led to compounds (**15–20**) of high H_2-antagonist affinity, which was related to the gastric antisecretory effect. Members of this structurally novel class of H_2 antagonists were 20- to 50-fold more potent than cimetidine both "in vitro" and "in vivo". Structure–activity relationships are discussed in terms of ionization properties, partitioning behavior, conformational aspects of the selected compound **17**, and of possible modes of interaction with the histamine H_2 receptor. It was found that the formamidine moiety was an important structural feature and that H_2-antagonist activity requires correct steric and electronic properties. Compound **17** (DA 4577), owing to its pharmacological profile and demonstrated safety in animals, was selected to be clinically investigated.

The development of cimetidine as a histamine H_2 receptor antagonist represents an excellent example of reasoned approach to the design of an antagonist modeled on an agonist effector molecule.

The therapeutic success of cimetidine stimulated the search for new histamine H_2 receptor antagonists following the knowledge of antagonist requirements accumulated during the research process on this therapeutic agent.

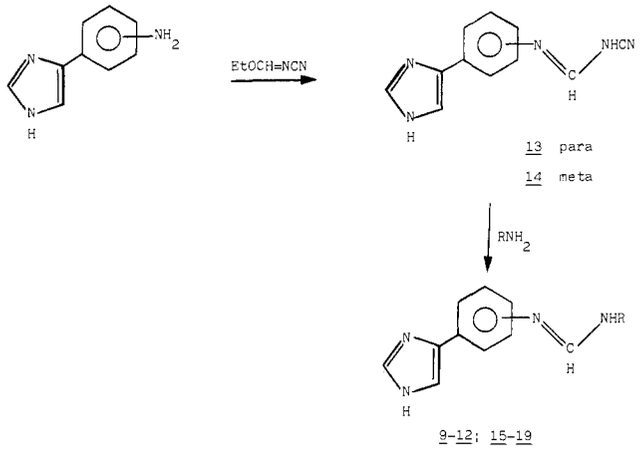
Ranitidine, tiotidine, etintidine, and oxmetidine can be considered as the first generation of H_2 antagonists. A second generation of newer compounds, e.g., SKF 93,479,[1] BL-6341A,[2] AH 22,216,[3] and YM 11,170,[4] claimed to be more potent or longer lasting than cimetidine, is now under study. Some of these compounds are nonimidazole structures, but all of them share structural features common to the prototype cimetidine molecule, i.e., a thiabutyl (or oxabutyl) side chain connecting a basic or basic substituted heteroaromatic or aromatic ring to a neutral moiety incorporating a 1,3 amidino system of NH groups.

This report describes a structurally novel class of potent H_2 receptor antagonists characterized by the presence of an amidino group positively charged at physiological pH (7.4), connected to an imidazole through a phenylene ring.

The importance of the basic amidino group and the involvement in receptor interaction of both the phenylene and the imidazole rings as reflected by H_2 antagonist activity in this series are also discussed.

Chemistry. The guanidine compounds (**1–4**, Table I) and the benzamidine compounds (**5–8**, Table I) were prepared by known procedures starting from the corresponding imidazolylanilines and imidazolylbenzonitriles,

Scheme I



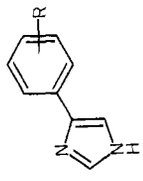
respectively. The formamidines (**9–12** and **15–19**, Tables I and II) were prepared by reaction of the corresponding (imidazolylphenyl)cyanoformamidines (**13** and **14**, Scheme I) with the appropriate amine according to the route depicted in Scheme I. The formamidine derivative **20**, which could not be prepared by this route, was prepared by reacting 4(5)-(4-aminophenyl)-1*H*-imidazole with ethyl *N*-*tert*-butylformimidate tetrafluoroborate in dichloro-

† This paper has been presented in part. See: "Abstracts of Papers", 186th National Meeting of the American Chemical Society, Washington, DC, Sept 1, 1983; American Chemical Society: Washington, DC, 1983; Abstr MEDI 62.

(1) Blakemore, R. C.; Brown, T. H.; Durant, G. J.; Ganellin, C. R.; Parsons, M. E.; Rasmussen, A. C.; Rawlings, D. A. *Br. J. Pharmacol.* **1981**, *74*, 200 P.
(2) Algieri, A. A.; Luke, G. M.; Standridge, R. T.; Brown, M.; Partyka, R. A.; Crenshaw, R. R. *Abstr. Pap. Am. Chem. Soc.* **1981**, *181*, MEDI 142.
(3) Brittain, R. T.; Daly, M. J.; Humphray, J. M.; Stables, R.; Ware, U. K. *Br. J. Pharmacol.* **1982**, *76* (Suppl N 2), 195 P.
(4) Takeda, M.; Takagi, T.; Yashima, Y.; Maeno, H. *Arzneim.-Forsch.* **1982**, *32*, 734.

0022-2623/84/1827-0380$01.50/0

Table I. (Imidazolylphenyl)guanidines, Imidazolylbenzamidines, and (Imidazolylphenyl)formamidines. Chemical and Biological Data



compd	R	position	mp, °C	crystn solvent	mol formula[a]	H_2 receptor antagonist act. (guinea pig atria)		acid antisecretory act.:[c]
						$K_B \times 10^{-8}$, [b] M	slope	ED_{50}, μg/kg, iv, rat (95% CL)
1	N=C(NH$_2$)$_2$	para	>280	EtOH	$C_{10}H_{11}N_5 \cdot 2HCl$	$>3 \times 10^{-5}$		
2	N=C(NHCH$_3$)NH$_2$	para	>280	MeOH-Et$_2$O	$C_{11}H_{13}N_5 \cdot 2HCl$	$>3 \times 10^{-5}$		
3	N=C(NH$_2$)$_2$	meta	240–245 dec	MeOH-Et$_2$O	$C_{10}H_{11}N_5 \cdot 2HCl$	550	1.04	3 200 (2700, 3900)
4	N=C(NHCH$_3$)NH$_2$	meta	280 dec	EtOH	$C_{11}H_{13}N_5 \cdot 2HCl$	440	0.78	3 600 (2700, 4900)
5	C(=NH)NH$_2$	para	>280	H$_2$O	$C_{10}H_{10}N_4 \cdot 2HNO_3$	$>3 \times 10^{-5}$		
6	C(=NCH$_3$)NH$_2$	para	>280	EtOH	$C_{11}H_{12}N_4 \cdot 2HCl$	$>3 \times 10^{-5}$		
7	C(=NH)NH$_2$	meta	>280	EtOH	$C_{10}H_{10}N_4 \cdot 2HCl$	720	0.59	>10 000
8	C(=NCH$_3$)NH$_2$	meta	>280	EtOH	$C_{11}H_{12}N_4 \cdot 2HCl$	870	1.05	3 500 (2400, 5100)
9	N=CHNH$_2$	para	169–171 dec	EtOH (95%)	$C_{10}H_{10}N_4 \cdot 2C_4H_4O_4$ [d]	3×10^{-5}		
10	N=CHNHCH$_3$	para	280 dec	EtOH	$C_{11}H_{12}N_4 \cdot 2HCl$	120	0.94	1 100 (750, 1600)
11	N=CHNH$_2$	meta	180–182 dec	EtOH	$C_{10}H_{10}N_4 \cdot 2C_4H_4O_4$ [d]	$>3 \times 10^{-5}$		
12	N=CHNHCH$_3$	meta	149–150 dec	EtOH	$C_{11}H_{12}N_4 \cdot 2C_4H_4O_4$ [d]	$>3 \times 10^{-5}$		
13	N=CHNHCN	para	234–236 dec	EtOH	$C_{11}H_9N_5$	$>3 \times 10^{-5}$		
14	N=CHNHCN	meta	206–207 dec	EtOH	$C_{11}H_9N_5$	$>3 \times 10^{-5}$		

[a] All compounds were analyzed for C, H, N, and Cl, when present. [b] K_B = dissociation constant calculated from the equation $K_B = B/DR - 1$, where DR represents the dose ratios of histamine required to produce half-maximal responses in the presence and absence of different concentrations (B) of compounds. [c] "In vivo" lumen perfused stomach of the anaesthetized, vagotomized rat, in which acid secretion was stimulated by histamine infusion [1 mg/(kg h)]. [d] Dimaleate salt.

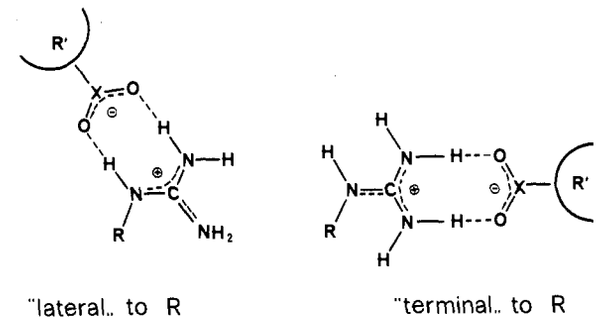


Figure 1. Hypothetical binding interactions of guanidinium cations (adapted from Walker[10] for a postulated anionic interaction of amidinium cations).

methane according to a method previously described by Weintraub et al.[5]

Results and Discussion

The Approach. In the course of their search for histamine H_2 receptor antagonists by chemical modification of the natural effector agonist, Durant and co-workers[6] succeeded in achieving an interesting lead, N^{α}-guanylhistamine. This compound proved to be a very weak inhibitor of histamine stimulation of gastric acid secretion, acting as a partial agonist at H_2 receptors. The successful elaboration that followed, the discovery of burimamide, metiamide, and, finally, cimetidine, is well known and extensively described in a review article by Ganellin and Durant.[7] In essence, these investigators dwelled on modifications of N^{α}-guanylhistamine by changing the ionization properties of the guanidine moiety and optimizing the imidazole ring tautomer population that was thought to be responsible for H_2 receptor interaction.

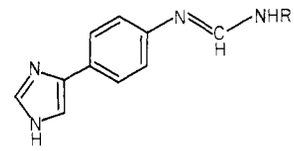
Our approach was complementary for the assumptions to that of Durant et al. but different in its development. We considered histamine and N^{α}-guanylhistamine as chemical starting points.

The guanidinium cation (Figure 1) of guanylhistamine may be viewed, differently from the tetrahedric ammonium group of histamine, as consisting of a double array of the planar amidine NHCNH system, in which the positive charge is completely delocalized over sp^2-hybridized atoms. The guanidinium group has more binding opportunities[8] compared to the ammonium group, due to the possibility of hydrogen bonding in preferred orientations according to the position of the amidine arrangements (Figure 1).[6,9]

We reasoned that N^{α}-guanylhistamine possessed an essential, albeit partial, requirement for H_2 antagonism, conferred by the peculiar structural characteristics of the guanidinium group, being insufficiently dissimilar from histamine to such a point that it could mimic its agonist effect. A structural element capable of effecting the desired differentiation was devised with the introduction of rigidity by suitable ring substitution of the ethane side chain. Physicochemical considerations concerning the favorable electronic and nonpolar properties that are involved in the possible charge-transfer or hydrophobic mode

(5) Weintraub, L.; Oles, S. R.; Kalish, N. *J. Org. Chem.* **1968**, *33*, 1679.
(6) Durant, G. J.; Parsons, M. E.; Black, J. W. *J. Med. Chem.* **1975**, *18*, 830.
(7) Ganellin, C. R.; Durant, G. J. In "Burger's Medicinal Chemistry", 4th ed., Part III; Wiley: New York, 1981; pp 487–551.
(8) Fastier, F. N. *Pharmacol. Rev.* **1962**, *14*, 37–90.
(9) Ganellin, C. R. *J. Med. Chem.* **1981**, *24*, 913.
(10) Walker, J. *J. Chem. Soc.* **1949**, 1996.

Table II. N-Substituted (Imidazolylphenyl)formamidine Histamine H_2 Antagonists



compd	R	mp, °C	crystn solvent	mol formula[a]	H_2 receptor antagonist act. (guinea pig atria) $K_B \times 10^{-8}$, M (±SE)[b]	slope (±SE)	acid antisecretory act.:[c] ED_{50}, μg/kg, iv, rat (95% CL)
15	C_2H_5	253–254 dec	EtOH	$C_{12}N_{14}N_4 \cdot 2HCl$	1.9 (0.53)	0.67 (0.06)	20 (16, 25)
16	n-C_3H_7	220–221 dec	EtOH	$C_{13}H_{16}N_4 \cdot 2HCl$	3.2 (0.54)	1.25 (0.05)	11 (9, 14)
17	i-C_3H_7	220–222 dec	i-PrOH (90%)	$C_{13}H_{16}N_4 \cdot 2HCl$	2.4 (0.40)	1.17 (0.05)	19 (16, 21)
18	$CH_2CH{=}CH_2$	152–153 dec	EtOH (95%)	$C_{13}H_{14}N_4 \cdot 2C_4H_4O_4$[d]	1.3 (0.22)	0.86 (0.04)	24 (20, 31)
19	i-C_4H_9	196–197 dec	EtOH	$C_{14}H_{18}N_4 \cdot 2HCl$	6.9 (1.94)	1.75 (0.12)	26 (20, 34)
20	t-C_4H_9	261–262 dec	MeOH	$C_{14}H_{18}N_4 \cdot H_2SO_4$	1.7 (0.33)[e]	1.07 (0.06)[e]	17 (14, 20)
cimetidine					47.0 (0.03)	1.03 (0.03)	560 (340, 900)

[a] All compounds were analyzed for C, H, N, and Cl, when present. [b] Footnote *b*, Table I. [c] Footnote *c*, Table I. [d] Dimaleate salt. [e] K_B obtained by measuring inhibition of inotropic effect induced by dimaprit in the electrically driven ventricular strip of the guinea pig.

of binding[11] favored the choice of the phenyl ring as a blocking moiety. Guanidino, amidino, and formamidino groups were chosen as guanidine-type moieties to assess the effect of the different orientation of the amidine NHCNH arrangements in the proposed bidentate interaction (Figure 1). Probe compounds were selected by considering combinations of the synthetically accessible meta- and para-substituted derivatives to determine the effect of internitrogen distance between the basic moieties and the imidazole ring, which was kept unaltered (Table I).[12]

In order to test the effect of substitution on the basic groups, it was deemed prudent to synthesize and test for H_2-antagonist activity unsubstituted and *N*-methyl-substituted derivatives and, on the basis of the results obtained, to expand the program to include higher homologue compounds.

Biological Results. The structures and results of biological testing in Table I illustrate the evolution of the structure–activity relationships within this series.

Compounds **1–14** were evaluated "in vitro" for histamine H_2 receptor antagonist activity by using the histamine-stimulated chronotropic response of the guinea pig atrium.[13] Gastric antisecretory activity was examined in the lumen perfused stomach of the anesthetized rat according to Gosh and Schild as modified by Lai.[14] Compounds that showed less than 50% inhibition of the histamine chronotropic effect at 3×10^{-5} M concentration were considered inactive and, therefore, not tested for antisecretory activity.

Of the guanidine derivatives (**1–4**), only the two meta-substituted derivatives (**3** and **4**) exhibited "in vitro" H_2-antagonist action accompanied by gastric acid antisecretory activity "in vivo". Also, the two meta-substituted benzamidine derivatives **7** and **8** were H_2 antagonists even though only **8** proved active in the "in vivo" assay. Of the formamidines (**9–12**), only compound **10** was found an active H_2 antagonist with a good gastric acid antisecretory activity. It is to be noted that differently from the two active guanidines (**3** and **4**) and benzamidines (**7** and **8**), the activity in the formamidine group was present only in the para *N*-methyl-substituted compound **10**. Only in the case of the latter did a further increase in the size of the substituent lead to a dramatic enhancement of both H_2-antagonist affinity and gastric acid antisecretory activity.

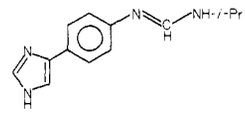
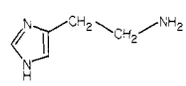
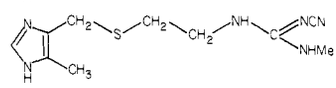
The results obtained on a limited series of N-substituted formamidines (Table II) demonstrate the potential of this structurally novel class of compounds.

As seen from the results in Table II, compounds **15–20** showed strong "in vitro" inhibitory activity toward histamine chronotropic responses, being several times (from 7 to 36) as potent as cimetidine, taken as a reference standard. The H_2-antagonist activity of the compounds was reflected in the inhibition of the "in vivo" gastric acid secretion evoked by histamine in the rat. Again the order of potency relative to cimetidine was 20–50 times.

In particular, compound **17**, which was selected for further studies, proved to be a competitive antagonist of

(11) Korolkovas, A. "Essentials of Molecular Pharmacology"; Wiley-Interscience: New York, 1970; pp 137–186.
(12) Active guanidino phenylene derivatives bearing a guanidinothiazole unit instead of the imidazole ring have been described previously (Gilman, D. J.; Jones, D. F.; Oldham, K.; Wardleworth, J. M.; Yellin, T. O. *Spec. Publ. R. Soc. Chem.* **1982**, no. 42. Recently, by: Hoffman, J. M.; Pietruszkiewicz, A. M.; Habecker, C. N.; Phillips, B. T.; Bolhofer, W. A.; Cragoe, E. J., Jr.; Torchiana, M. L.; Lumma, W. C., Jr.; Baldwin, J. J. *J. Med. Chem.*, **1983**, *26*, 140). Interestingly, H_2-antagonist activity proved in these compounds to be dependent on the *m*-phenylene position.
(13) Black, S. W.; Duncan, W. A. M.; Durant, G. J.; Ganellin, C. R.; Parsons, M. E. *Nature (London)* **1972**, *236*, 385.
(14) Lai, K. S. Gut **1964**, *5*, 327.
(15) Albert, A.; Serjeant, E. P. "Ionization Constants of Acids and Bases"; Methuen & Co.: London; 1981, pp 1–68.
(16) Synthetic procedures for N^{τ}-methyl- and N^{π}-methylimidazole derivatives are described in European Patent Application 83200690.2, 1983.
(17) Ganellin, C. R. *J. Pharm. Pharmacol.* **1973**, *25*, 787.
(18) Durant, G. J.; Emmet, J. C.; Ganellin, C. R.; Miles, P. D.; Parsons, M. E.; Prain, H. D.; White, G. R. *J. Med. Chem.* **1977**, *20*, 901.

Table III. Apparent pK_a Values at 25 °C and Mole Percentages of Species at pH 7.4 of Compound 17, Histamine, and Cimetidine

compd	structure	pK_a		mole % at pH 7.4		
		imidazole	end group	dication	monocation	neutral species
17		5.58[a]	8.88[a]	1.49	88.63 (N^{τ}-H)[b] 6.67 (N^{π}-H)[b]	3.21
histamine		6.07[c]	9.81[c]	4.47[d]	76.12 (N^{τ}-H)[d] 19.02 (N^{π}-H)[d]	0.39[d]
cimetidine		6.80[e]	−0.4[f]		20.08[g]	79.92[g]

[a] Determined by potentiometric titration of 17·2HCl, H_2O solvent; see ref 15. [b] The relative population of N^{τ}-H vs. N^{π}-H of imidazole was determined by pK_a comparison in H_2O solution of the corresponding *N*-methylimidazole derivatives[16] (N^{τ}-Me, pK_a = 5.39; N^{π}-Me, pK_a = 6.51; K_t = 13.18) of 17 according to the method used by Ganellin for histamine; see ref 17. [c] Literature values.[17] [d] Calculated from the pK_a values reported in ref 17. [e] Literature value.[18] [f] Proton gained referred to cyanoguanidine.[18] [g] Calculated from the pK_a value 6.80.

histamine at H_2 sites so far examined. It was also shown to possess a very high degree of selectivity for H_2 receptors, as it failed to interact with histamine H_1, muscarinic, nicotinic, 5-HT, or β receptors at concentrations 1000-fold higher than those required to fully inhibit H_2-mediated responses.

Besides the antisecretory activity demonstrated in the anesthetized rat (Table II), compound 17 suppressed acid secretion in the conscious dog with Heidenhain pouch. In this species, the ED_{50} (po) of 17 was 68 μg/kg in antagonizing peak acid secretion evoked by nearly maximal challenge with histamine. This value well compares with the ED_{50} by the intravenous (iv) route (24 μg/kg), indicating that compound 17 is well absorbed.

Discussion. Examination of the results reported in Tables I and II shows an enhancement in activity passing from the *N*-methyl-substituted derivative 10 to higher homologues in the formamidine series. A number of differently N-substituted (imidazolylphenyl)formamidines have been prepared, and the results obtained indicated that, although the R substituent is not critical for expression of activity, the presence of a non-electron-withdrawing group with a certain steric bulk is necessary. When instead an electronegative substituent is introduced and a neutral amidine species is obtained (e.g., cyanoformamidine 13, Table I), the H_2 antagonism is completely abolished. This suggests that a positively charged amidino group is required for activity.

Compound 17, which was selected for detailed studies, exhibits ionization properties similar to those of histamine and different from cimetidine (Table III). pK_a values of 5.58, due to the loss of a proton from the protonated imidazole ring, and 8.88, due to the loss of a proton from the amidine cation, indicate that compound 17 exists at physiological pH (7.4) as a mixture of ionic and neutral species in their possible tautomeric forms (Scheme II). In contrast to cimetidine, which is approximately 20% ionized at the imidazole ring, and similarly to histamine, the monocation of 17 is the predominant species (95.30%, taken as a sum of the N^{π}-H and N^{τ}-H tautomers) (Scheme II, Table III). It is likely that this monocation is the active form at the histamine H_2 receptor.

When this critical component is focused upon, it is of interest to consider that protonation on the imino nitrogen of the amidine group results in the resonance-stabilized amidinium cation, where the positive charge is delocalized over a plane of sp^2-hybridized atoms. It should also be noted that this cation may exist in four different planar

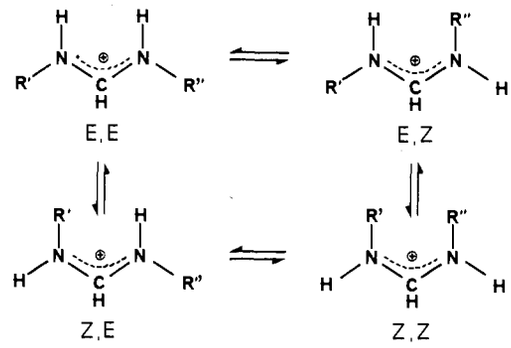


Figure 2. Possible planar conformation of the amidinium cation of compound 17 in solution [R′ = 4-(4-imidazolyl)phenyl; R″ = *i*-Pr].

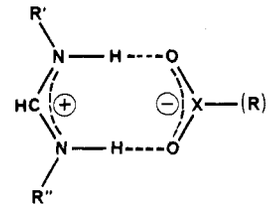
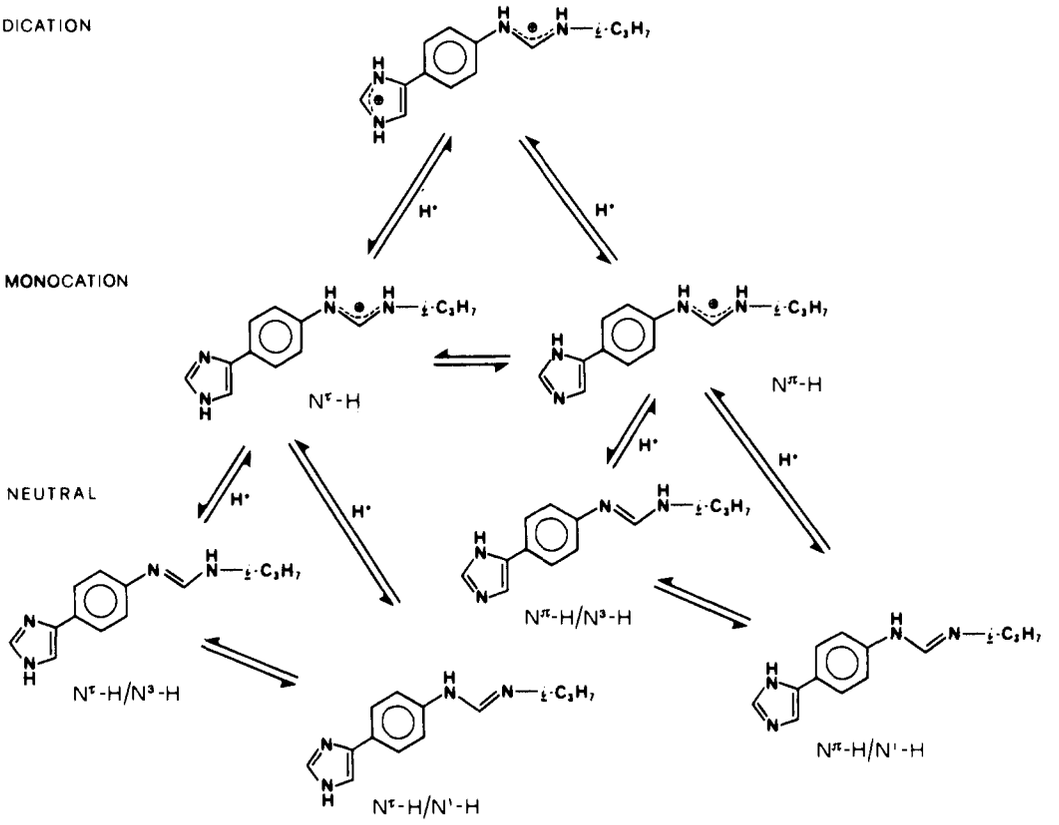


Figure 3. Proposed binding interaction of compound 17 to an anionic site of the histamine H_2 receptor [X = C, P; (R) = receptor; R′ = *i*-Pr; R″ = 4-(4-imidazolyl)phenyl].

conformations (Figure 2). NMR studies[19] performed on compound 17, which are in agreement with literature data on similar compounds,[20] have shown that only the *E,E* and *E,Z* forms are present in solution, the other two forms (*Z,E* and *Z,Z*) being unfavorable due to internal steric interaction. An activation energy[21] of 16.07 kcal/mol obtained for the thermal isomerization *E,E*⇌*E,Z* in D_2O solution indicates that these two conformational isomers are freely interconvertible (Figure 2). On the basis of these findings, we suggest that the amidinium cation of 17 binds to anionic sites of the histamine H_2 receptor through a electrostatic interaction supplemented by hydrogen bonds. A similar

(19) Bazzano, C.; Vanoni, P. C.; Gallazzi, A., manuscript in preparation.
(20) Wellman, K. M.; Harris, D. L. *Chem. Commun.* **1967**, 256.
(21) Calculated by ^1H NMR from the coalescence temperature (T_c = 35 °C) of the two singlets (δ 8.11, 7.97) due to the methine proton of the formamidine group of 17·2HCl in D_2O (pH 7.18, phosphate buffer) ($\Delta\nu$ = 11.2 Hz, 27 °C) using the Eyring equation $\Delta G^{\ddagger} = 4.57T_c(10.32 \log T_c/K_c)$, where $K_c = \pi\Delta\nu/2^{1}/_{2}$.

Scheme II. Ionic and Tautomeric Species of Compound 17



hypothesis has been advanced by Walker[10] for amidine bonding interactions.

In this complex, the nitrogen atoms of the charged amidine system in the *E,E* conformation and the oxygen atoms of a hypotetical carboxylate or phosphate anion would be coplanar and assume a relatively rigid configuration of minimum potential energy (Figure 3). Since free interconversion occurs between the *E,E* and the *E,Z* conformations, the tightly binding conformer (*E,E*) would be conformationally selected in receptor binding. The phenyl ring and the isopropyl group in **17** would bind to lipophilic accessory binding sites of the receptor, while the imidazole ring, which results practically uncharged at physiological pH (7.4) (Table III), would provide additional attachment through acceptor–donor H bondings.

Partitioning properties of **15** are clearly different from those of histamine and cimetidine. The rather low $P_{octanol}$ value[22] (3.7) of **17** at pH 7.4 justifies the large amount of the amidinium cation present at this pH, whereas the $P_{octanol}$ value (117) calculated for the neutral molecule[23] accounts for the lipophilicity conferred by the phenylene ring and the isopropyl group (histamine $P_{octanol}$ = 0.2 at pH 11.8;[24] cimetidine $P_{octanol}$ = 2.5 at pH 9.2[18]).

Comparison of **17** with histamine shows that both molecules possess an imidazole ring and a cationic head of comparable pK_a values (Table III). The most relevant difference is the presence in **17** of a phenylene ring in place of the ethane chain of histamine. It would be tempting to speculate that histamine and (imidazolylphenyl)formamidines share common receptor binding sites in receptor interaction. For the latter class, two degrees of freedom are possible around the planar phenylene ring, so that the imidazole and the amidinium cation may be planar or twisted with respect to the aromatic ring. This places limits on the internitrogen distances. Comparison of the distance between the amidine N^1 and the imidazole N^π in **17** (~6.7-Å minimum achievable distance, Dreiding models) with that of histamine (NH_2–imidazole N^π distance of 5.1 Å, calculated for the fully extended formation[25]) rules out topographic overlapping of common functional groups. Conceivably, **17** could share a binding feature common with histamine; i.e., the amidine moiety could bind to the anionic site of histamine, the other moieties providing enhanced blockade of the receptor portion normally occupied by histamine, or bind to accessory areas.

Comparison of **17** with cimetidine may be only speculative due to the considerable degrees of rotational freedom about the flexible four-membered chain of the latter. Cimetidine possesses, like (imidazolylphenyl)formamidines, an imidazole ring and a masked amidine system (cyanoguanidine), which, however, is neutral. By considering the ionization properties of **17**, where the imidazole is practically uncharged at pH 7.4 (Table III), one could relate the imidazole ring of **17** to the neutral amidine arrangement present in the cyanoguanidine of cimetidine and relate the basic amidine group of **17** to the partially charged imidazole of cimetidine.

In conclusion, (imidazolylphenyl)formamidines represent a structurally novel class of potent H_2 receptor antagonists that exhibit strong gastric antisecretory activity in different animal models. This activity appears to be related to the H_2-inhibiting properties. Members of this series are under active development to assess their therapeutic potential

(22) 1-Octanol partition coefficient, aqueous buffer (pH 7.4), 26 °C, shaker flask method. Martin, Y. C. In "Quantitative Drug Design", Marcel Dekker: New York and Basel, 1978; pp 62–76.

(23) Calculated from the observed P_{app} (pH 7.4) of **17** and corresponding pK_a by the following relationship: $P = P_{app}/1 - \alpha$. See ref 22.

(24) Ganellin, C. R.; Parsons, M. E. In "Pharmacology of Histaminic Receptors"; Wright PSG: Bristol, London, and Boston, 1982; p 18.

(25) Ganellin, C. R. *J. Med. Chem.* **1973**, *16*, 620.

as gastric antisecretory agents. In particular, compound 17 (DA 4577), on the basis of its pharmacological profile and demonstrated safety in animals, was selected to be evaluated in man.

Experimental Section

Chemistry. All melting points are uncorrected and were taken on a Büchi capillary melting point apparatus. Proton NMR spectra were recorded on Varian Associates Models T-60 and CFT-20 spectrometers in the indicated solvent. Chemical shifts are reported as δ values relative to tetramethylsilane as internal standard. Elemental analyses were performed on all compounds, and the analytical values were within ±0.4% of the theoretical values.

General Method for the Preparation of (Imidazolylphenyl)guanidines (Table I, 1–4). *N*-Methyl-*N'*-(3-1*H*-imidazol-4-ylphenyl)guanidine Dihydrochloride (4). 4(5)-(3-Aminophenyl)-1*H*-imidazole[26] (21.8 g, 0.137 mol) was added to a solution of dimethyl cyanothioimidocarbonate[27] (20 g, 0.137 mol) in anhydrous ethanol (110 mL), and the mixture was stirred at room temperature for 5 days. The clear solution was evaporated to dryness to give *N*-cyano-*S*-methyl-*N'*-(3-1*H*-imidazol-4-ylphenyl)guanidine (34.5 g). This was added to a stirred solution of 30% methylamine in ethanol (280 mL) and cooled in an ice bath. The solution was allowed to warm gradually to room temperature overnight. The solvent was evaporated, and the oily residue was crystallized from diethyl ether to give 28.5 g of *N*-cyano-*N'*-methyl-*N''*-(3-1*H*-imidazol-4-ylphenyl)guanidine, mp 115-117 °C. A solution of this *N*-cyanoguanidine (28 g, 0.116 mol) in concentrated hydrochloric acid was refluxed for 10 h, cooled at room temperature, and evaporated to dryness. The crude guanidine so obtained was purified through its picrate salt in water, from which the corresponding hydrochloride was conventionally restored. Recrystallization from ethanol furnished 19.2 g of pure 4: overall yield 48%.

Compound 2 was similarly prepared in 51% yield starting from 4(5)-(4-aminophenyl)-1*H*-imidazole.[28]

Compounds 1 and 3 were prepared following the same method in 38 and 42% yields, respectively, with 30% ammonium hydroxide instead of methylamine.

The NMR data for 4 were typical: NMR (Me_2SO-d_6–$CDCl_3$) δ 2.93 (d, 3 H, CH_3, J = 4 Hz), 7–8.3 (m, 4 H, aromatic H), 8.16 (s, 1 H, CH=C), 9.2 (s, 1 H, HNC*H*=N).

General Method for the Preparation of Imidazolylbenzamidines (Table I, 5–8). 4-1*H*-Imidazol-4-ylbenzamidine Dinitrate (5). A mixture of 4(5)-(4-cyanophenyl)-1*H*-imidazole[29] (3 g, 17.7 mmol) and thiourea (6.7 g, 88 mmol) was heated at 150 °C for 5 h. The cooled mixture was suspended in water, acidified with 20% aqueous HNO_3 (pH 2), and filtered. The clear solution was cooled to afford 1.8 g (34% yield) of a crystalline precipitate consisting of pure 5.

Compound 6 was similarly obtained in 29% yield with *N*-methylthiourea. Compounds 7 and 8 were analogously prepared starting from 4(5)-(3-cyanophenyl)-1*H*-imidazole and the appropriate thiourea.

4(5)-(3-Cyanophenyl)-1*H*-imidazole was synthesized by reacting α-bromo-3-cyanoacetophenone[30] (33.9 g, 0.151 mol) and formamide (85.2 g) in the presence of water (8 mL) at 140 °C for 4 h following the conditions reported in ref 29 for 4(5)-(4-cyanophenyl)-1*H*-imidazole: mp 138–140 °C; 60% yield.

The NMR data for 5 were typical: NMR (Me_2SO-d_6–$CDCl_3$) δ 8.04 (s, 4 H, aromatic H), 8.33 (s, 1 H, CH=C), 9.25 (s, 1 H, NHC*H*=N).

General Method for the Preparation of (Imidazolylphenyl)formamidines (Table I, 9–12; Table II, 15–19). *N*-Cyano-*N'*-(4-1*H*-imidazol-4-ylphenyl)formamidine (13, Table I). A solution of 4(5)-(4-aminophenyl)-1*H*-imidazole (50.9 g, 0.32 mol) and ethyl *N*-cyanoformimidate[31] in ethanol (500 mL) was stirred at room temperature overnight. The product that crystallized out was collected by filtration to give 60 g (88% yield) of the desired 13: NMR (Me_2SO-d_6–$CDCl_3$) δ 7.48 (s, 1 H, CH=C), 7.72 (s, 1 H, imidazole NHC*H*=N), 7.74 (d, 2 H, aromatic H, J = 8.4 Hz), 7.29 and 7.65 (2 d, 2 H, aromatic H, J = 8.4 Hz), 9.04 (s, 1 H, amidine NHC*H*=N).

***N*-Cyano-*N'*-(3-1*H*-imidazol-4-ylphenyl)formamidine (14, Table I)** was similarly prepared, starting from 4(5)-(3-aminophenyl)-1*H*-imidazole in 85% yield.

***N*-Isopropyl-*N'*-(4-1*H*-imidazol-4-ylphenyl)formamidine Dihydrochloride (17).** Isopropylamine (235.8 g, 3.99 mol) was quickly added to a suspension of *N*-cyano-*N'*-(4-1*H*-imidazol-4-ylphenyl)formamidine (120 g, 0.57 mol) in water (170 mL). A solution was obtained, from which, after few minutes, a solid separated, which was washed with water and collected by filtration. The crude base so obtained was dissolved in 90% aqueous 2-propanol, and gaseous HCl was passed through this solution to precipitate the crystalline dihydrochloride of 17 (102.8 g, 60% yield).

Compounds 9–12, 15, 16, 18, and 19 were analogously prepared, starting from the appropriate *N*-cyanoformamidine derivative 13 or 14 and the requisite amine. All compounds were purified and characterized through their salts as indicated in Tables I and II.

The NMR data for 17, as a base, were typical: NMR (Me_2SO-d_6–$CDCl_3$) δ 1.17 (d, 6, CH_3, J = 6.5 Hz), 3.98 [br m, 1, $(CH_3)_2CH$], 6.88 (d, 2 H, aromatic H, J = 7.4 Hz), 7.57 (d, 2 H, aromatic H, J = 7.4 Hz), 7.31 (s, 1 H, CH=C), 7.62 (s, 2H, imidazole and amidine NHC*H*=N).

***N*-*tert*-Butyl-*N'*-(4-1*H*-imidazol-4-ylphenyl)formamidine Sulfate (20).** A solution of freshly prepared triethyloxonium fluoroborate (9.5 g, 0.05 mol) in dichloromethane (20 mL) was dropped at 0 °C into a solution of *tert*-butylformamide (5.1 g, 0.05 mol) in dichloromethane (50 mL). The clear solution was allowed to stand at room temperature overnight and cooled again at 0 °C. A solution of 4(5)-(4-aminophenyl)-1*H*-imidazole (4 g, 25 mmol) in anhydrous ethanol was slowly dropped in at 0–5 °C, and the reaction mixture was stirred at room temperature for 8 h. After evaporation to dryness, the solid residue was dissolved in water, and the aqueous solution was basified (pH 9) with 10% sodium hydroxide. The oily product that separated was extracted with ethyl acetate. The organic layer was washed with water, dried ($MgSO_4$), and evaporated to dryness to give a thick oil. This oil was dissolved in methanol and acidified with 30% aqueous sulfuric acid to give the sulfate 20, as a white solid (4.7 g, 55% yield): NMR (Me_2SO-d_6–$CDCl_3$) δ 1.5 [s, 9 H, $(CH_3)_3C$], 7.53 (d, 2 H, aromatic H, J = 8.5 Hz), 7.9 (d, 2 H, aromatic H, J = 8.5 Hz), 8.02 (s, 1 H, CH=C), 8.7 (br s, 1, amidine NHC*H*=N), 9.16 (s, 1 H, imidazole NHC*H*=N).

Pharmacology. All compounds were administered as their water-soluble salts, and the ED_{50} values are expressed in terms of the effective content of base. (Imidazolylphenyl)formamidines were found to decompose slowly in aqueous solution, especially at alkaline pH's, to the corresponding amino and formamido derivatives. The compounds proved stable for the period of time and conditions required for pharmacological investigation, as checked by careful TLC investigation.

Histamine H_2 Antagonist Activity. The procedure employed was that described by Black et al.,[13] with some modifications. Guinea pigs were killed by cervical dislocation and exanguinated. The atria were dissected free and placed in a 50-mL organ bath at 32 °C containing oxygenated (95% O_2/5% CO_2) Krebs–Henseleit buffer (pH 7.4). Rate and force were measured with a transducer exerting an initial tension of 1 g. Tissues were equilibrated for 1 h before a cumulative concentration–response curve to the chronotropic effect of histamine was obtained. When the effect of antagonists was assessed, a second concentration–response curve was repeated in the presence of successive concentrations of the antagonist, which had been present for 30 min in the bath. Dose ratios (DR) were calculated as the ratio of histamine concentrations required to produce one-half of maximal stimulation in the presence and absence of antagonist concentration (B). Dissociation constants (K_b) were calculated by the

(26) Schunack, W. *Arch. Pharm. (Weinheim, Ger.)* **1973**, *306*, 934.
(27) Hantzch, A.; Wolvekamp, M. *Justus Liebigs Ann. Chem.* **1904**, *331*, 265.
(28) Balaban, E. *J. Chem. Soc.* **1925**, 2701.
(29) Ellis, G. P.; Epstein, C.; Fitzmaurice, C.; Golberg, L.; Lord, G. H. *J. Pharm. Pharmacol.* **1964**, *16*, 400.
(30) Griszkiewicz-Trochimowsky, E.; Schmidt, W.; Griszkiewicz-Trochimowsky, O. *Bull. Soc. Chim. Fr.* **1948**, 593.
(31) Huffman, K. R.; Schaefer, F. C. *J. Org. Chem.* **1963**, *28*, 1816.

procedure described by Waud.[32]

Gastric Acid Antisecretory Activity. Compounds were evaluated in anesthetized rats with the lumen perfused stomach preparation[33,14] and for some detailed studies in dogs equipped with well-established Heidenhain pouches. Secretion was elicited in male rats (Sprague–Dawley; Charles River, 250 g) anesthetized with urethane (1 g/kg), by constant intravenous infusion of histamine [1 mg/(kg h)]. As the stimulated acid secretion reached a steady output, compounds were injected intravenously in a series of increasing doses until the secretion was maximally suppressed. ED_{50} values were calculated from the regression lines representing percent inhibition of acid output. Histamine [60 μg/(kg h)] was also employed to stimulate acid secretion in conscious dogs. Compounds were administered by bolus intravenous injection when the stimulated acid output had stabilized. Secretion was followed by draining the pouch content at 15-min intervals and following the return of acid output to predrug levels. The dose of each compound inhibiting secretion by 50% (ED_{50}) was calculated from the regression line representing percent inhibition.

Selectivity Studies. Receptor selectivity was assessed by "in vitro" experiments on guinea pig ileum in which contractions were elicited by histamine (H_1 receptors), acetylcholine (muscarinic), nicotine (nicotinic), or 5-hydroxytryptamine (5-HT receptors). Interference with β-adrenoceptors was evaluated in the isolated guinea pig atrium stimulated by isoproterenol.

Registry No. 1, 88304-42-1; 1 (base), 88304-59-0; 2, 88304-43-2; 2 (base), 88304-60-3; 3, 88304-44-3; 3 (base), 88304-61-4; 4, 88304-45-4; 4 (base), 88304-62-5; 5, 88304-47-6; 5 (base), 88304-46-5; 6, 88304-48-7; 6 (base), 88304-63-6; 7, 88304-49-8; 7 (base), 88304-64-7; 8, 88304-50-1; 8 (base), 88304-65-8; 9, 88304-52-3; 9 (base), 88304-51-2; 10, 83184-18-3; 10 (base), 83184-12-7; 11, 88304-54-5; 11 (base), 88304-53-4; 12, 83184-37-6; 12 (base), 83184-36-5; 13 (base), 83184-32-1; 14 (base), 83184-33-2; 15, 83184-41-2; 15 (base), 83184-40-1; 16, 83184-49-0; 16 (base), 83184-48-9; 17, 83304-55-6; 17 (base), 83184-43-4; 18, 83184-46-7; 18 (base), 83184-45-6; 19, 83184-53-6; 19 (base), 83184-52-5; 20, 88304-66-9; 20 (base), 83184-14-9; $C_2H_5NH_2$, 75-04-7; n-$C_3H_7NH_2$, 107-10-8; 1-$C_3H_7NH_2$, 75-31-0; CH_2=$CHCH_2NH_2$, 107-11-9; i-$C_4H_9NH_2$, 78-81-9; t-$C_4H_9NH_2$, 75-64-9; 4(5)-(3-aminophenyl)-1H-imidazole, 83184-01-4; dimethyl cyanothioimidocarbonate, 67434-79-1; N-cyano-S-methyl-N'-(3-1H-imidazol-4-ylphenyl)-guanidine, 88304-56-7; N-cyano-N'-methyl-N''-(3-1H-imidazol-4-ylphenyl)guanidine, 88304-57-8; 4(5)-(4-aminophenyl)-1H-imidazole, 29528-28-7; 4(5)-(4-cyanophenyl)-1H-imidazole, 34443-07-7; thiourea, 62-56-6; N-methylthiourea, 598-52-7; 4(5)-(3-cyanophenyl)-1H-imidazole, 88304-58-9; α-bromo-3-cyanoacetophenone, 50916-55-7; formamide, 75-12-7; ethyl N-cyanoformimidate, 4428-98-2; *tert*-butylformamide, 2425-74-3.

(32) Waud, D. R. *Methods Pharmacol.* **1976**, *3*, 471.
(33) Gosh, R. N.; Schild, H. O. *Br. J. Pharmacol. Chemother.* **1958**, *13*, 54.

6-Hydroxy-4-[2-(di-*n*-propylamino)ethyl]indole: Synthesis and Dopaminergic Actions

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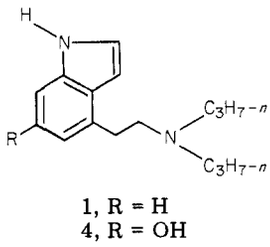
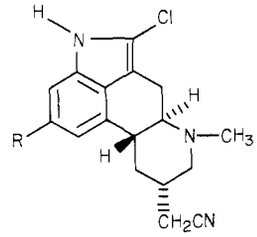
The title compound was proposed to be a biologically active metabolite of a dopaminergic agent, 4-[2-(di-*n*-propylamino)ethyl]indole. This proposed metabolite was synthesized by a multistep sequence beginning with methyl 3,5-dinitro *o*-toluate, and involving the Batcho–Leimgruber modification of the Reissert indole synthesis. The target compound exhibited high potency/activity in vivo in a cat cardioaccelerator nerve assay and in vitro in an isolated cat atrium assay. It manifested maximal pharmacological effect less than 5 min after intravenous administration in cats, as compared with a 20-min lag time following intravenous administration of the nonoxygenated congener. These pharmacological data are consistent with the proposal that the target compound is a metabolite of 4-[2-(di-*n*-propylamino)ethyl]indole.

Prior communications[1-3] have described prominent dopaminergic agonist actions of a simple indole derivative, **1**, designed as a fragment of the dopaminergic ergoline



1, R = H
4, R = OH



2, R = H
3, R = OH

derivative lergotrile (**2**). The slow (30–40 min) onset of pharmacological effects following intravenous administration of **1** in intact animals, coupled with its dopaminergic inactivity in an in vitro assay using isolated cat atrium and its very weak binding activity in calf caudate tissue,[2] led to the proposal that **1** may be metabolically activated in vivo. Wong and Bymaster[4] reported that 13-hydroxylergotrile (**3**), a metabolite of lergotrile, had greater affinity than lergotrile in a dopamine agonist receptor binding assay. Parli et al.[5] reported that 13-hydroxylergotrile is 100 times more active than lergotrile in vitro in inhibiting prolactin release from the anterior pituitary. In the present work, it was speculated that the indole system **1** might be metabolically hydroxylated in a position and analogous to that of the 13-position of lergotrile, namely, the 6-position (structure **4**). In support of this speculation are reports that rabbit liver microsomes hydroxylate tryptamine, indole-3-acetic acid, and related indoles in the 6-position[6] and that tryptamine and in-

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(1) Cannon, J. G.; Demopoulos, B. J.; Long, J. P.; Flynn, J. R.; Sharabi, F. M. *J. Med. Chem.* **1981**, *24*, 238.
(2) Cannon, J. G.; Long, J. P.; Demopoulos, B. J. *Adv. Biosci.* **1982**, *37*, 189.
(3) Cassady, J. M.; Clemens, J. A. U.S. Patent 4 378 368, 1983; *Chem. Abstr.* **1983**, *99*, 16566.
(4) Wong, D. T.; Bymaster, F. P. "Abstracts of Papers", Joint Central-Great Lakes Regional Meeting of the American Chemical Society, Indianapolis, IN, 1978; American Chemical Society: Washington, DC, 1978; Abstr MEDI 25.
(5) Parli, J; Schmidt, B.; Shaar, C. J. *Biochem. Pharmacol.* **1978**, *27*, 1405.
(6) Jepson, J. B.; Zaltzman, P.; Udenfriend, S. *Biochim. Biophys. Acta* **1962**, *62*, 91.

0022-2623/84/1827-0386$01.50/0