N-(Fluoroethyl)(imidazolylphenyl)formamidines. The Issue of the Active Species of Mifentidine

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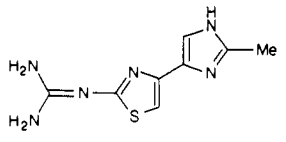
Three *N*-fluoroethyl-substituted (imidazolylphenyl)formamidine derivatives, namely, 2-fluoroethyl (**3b**), 2,2-difluoroethyl (**3c**), and 2,2,2-trifluoroethyl (**3d**), were prepared to test the effect of fluorine substitution on basicity and, then, on H_2-antagonist affinity in comparison with the unsubstituted *N*-ethyl derivative (**3a**), taken as a model of mifentidine. Imidazolylphenyl isothiocyanate (1), obtained by reaction of 4-(aminophenyl)imidazole with carbon disulfide and ethyl chloroformate, was condensed with the requisite 2-fluoro-substituted ethylamines to give the intermediate thioureas (**2b–d**). Desulfurization of these thioureas by Raney nickel furnished the desired formamidines (**3b–d**). Increasing fluorine substitution was found to decrease basicity of the formamidino group substantially (**3a**, pK_a = 8.65; **3b**, pK_a = 8.12; **3c**, pK_a = 6.60; **3d**, pK_a = 6.14), while having a modest effect on the imidazole portion. Affinity at the H_2 receptors, evaluated from antagonism of histamine-stimulated chronotropic response on guinea pig atria, increased following fluorine substitution (**3a**, K_B = 177; **3b**, K_B = 61; **3c**, K_B = 21; **3d**, K_B = 7.6). It is concluded that H_2-receptor antagonist affinity in the mifentidine series is mostly dependent on the availability of the neutral species. These data support the hypothesis that mifentidine, like cimetidine, acts through the neutral species.

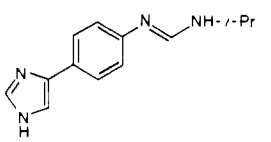
According to a chemical differentiation between H_1 and H_2 histamine receptor antagonists, first proposed by Ganellin and co-workers,[1] anti H_1 drugs are lipophilic molecules that bear similarity to histamine in possessing a positively charged ammonium side chain, while H_2 antagonists are rather hydrophilic molecules that resemble histamine in having an imidazole, but differ in the side chain which is neutral and polar.

Even though this simplified distinction was based on few representatives of the novel class, i.e. burimamide and metiamide, it provided a valid guideline for subsequent molecular manipulations. An imidazole ring, a thiabutyl chain, and a neutral polar moiety are the molecular determinants for antagonist activity commonly present in the archetypal structures of H_2-receptor antagonists, including cimetidine, the first compound entered into clinical use. However, the imidazole ring, initially assumed as an essential structural requirement for H_2 antagonism, could be successfully replaced by other moieties possessing distinct structural or physical chemical characteristics, e.g. [(dimethylamino)methyl]furan in ranitidine[2] or (piperidinomethyl)benzene in lamtidine[3] and several derivatives. On the contrary, the fundamental structural features of the neutral polar moiety, consisting in nonbasic linear or cyclic amidine systems, were essentially retained throughout the numerous series of H_2-receptor antagonists that have thus far appeared (Figure 1).[4]

A notable departure from classical structures is represented by the conformationally restricted H_2-receptor antagonists, zaltidine (CP 57,361-1) and mifentidine (DA 4577). Zaltidine[5] contains two weakly basic centers [pK_a = 5.5 (guanylthiazole) and pK_a = 6.7 (2-methylimidazole)]. It was suggested that the 2-methylimidazole and the 2-guanylthiazole mimic, respectively, the cyanoguanidine and the imidazole of cimetidine. This apparent bioisosteric pattern and associated pK_a values would imply that zaltidine interacts with the H_2 receptor in a classical way, i.e. through its neutral form.



zaltidine (CP 57, 361-1)



mifentidine (DA 4577)

Mifentidine[6,7] still possesses an amidine system in the form of a formamidino group, but unlike zaltidine and the other H_2-receptor antagonists, its pK_a values [pK_a = 5.58 (imidazole), pK_a = 8.88 (formamidine)] and calculated percent ionizations point to the monocationic form as the predominant species (95.30%) in aqueous solution at pH 7.4 (Table III). On the basis of these properties, a mode of binding for mifentidine was advanced by us, in which the charged molecule was suggested as the active species at the H_2 receptor. This model has been questioned by Haaksma et al.[8] on the ground of affinity estimates for the H_2 receptor performed with mifentidine at different pH values. These authors concluded that, like cimetidine, mifentidine interacts with the H_2 receptor through the neutral species.

The aim of the present study was to investigate the issue of the active species of mifentidine by using a different approach. Therefore, the effect on H_2-receptor affinity of three mifentidine analogues endowed with intrinsic decreasing basicity was determined in comparison with a model molecule. Reduction in basicity was attained by introducing in a specific position of the mifentidine pharmacophore suitable groups selected so as to avoid interference with other critical parameters.

The results of this study are presented, and an interpretation for the interaction with the H_2 receptor of am-

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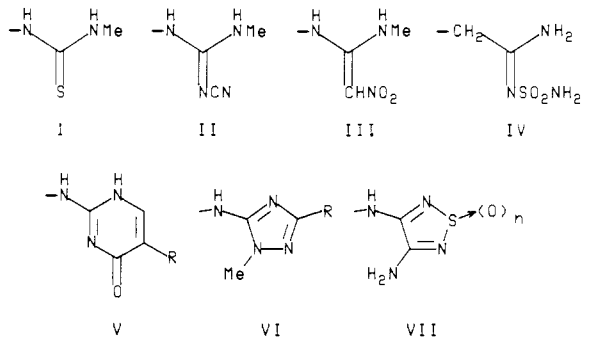
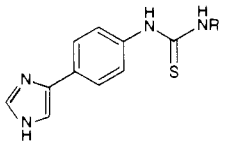


Figure 1. Neutral polar moieties recurring in histamine H_2 receptor antagonists. I: burimamide, metiamide. II: cimetidine, tiotidine. III: ranitidine, nizatidine. IV: famotidine. V: oxmetidine, lupitidine, donetidine. VI: lamtidine, loxtidine, sufotidine. VII: BL-6341, BMY-25405, BMY-25271.

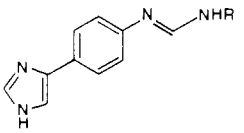
Table I. *N*-(Fluoroethyl)-*N'*-(4-imidazol-4-ylphenyl)thioureas



compd	R	mp,[a] °C	yield, %	cryst solv	mol formula[b]
2b	FCH_2CH_2	129–131	90	AcOEt	$C_{12}H_{13}FN_4S$[c]
2c	F_2CHCH_2	165–167	78	CH_2Cl_2	$C_{12}H_{12}F_2N_4S$
2d	F_3CCH_2	183–185	90	CH_2Cl_2	$C_{12}H_{11}F_3N_4S$

[a] Purity of the compounds was checked by TLC using solvent system A. [b] All compounds were analyzed for C, H, N, and S. [c] C: calcd, 54.53; found, 53.96.

Table II. *N*-(Fluoroethyl)-*N'*-(4-imidazol-4-ylphenyl)formamidines

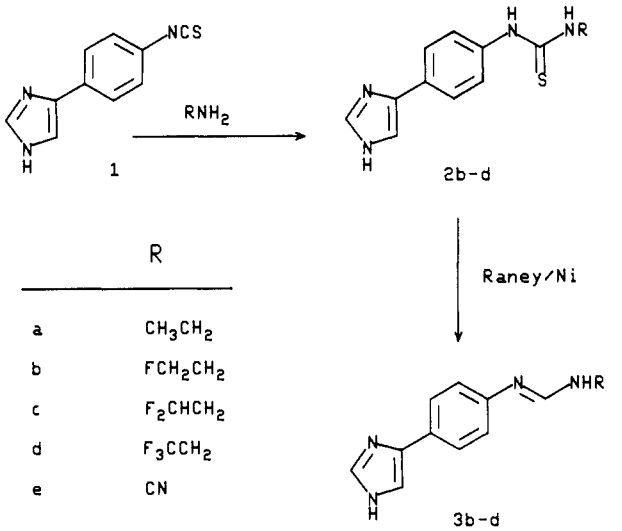


compd	R	mp,[a] °C	yield, %	cryst solv	mol formula[b]
3b	FCH_2CH_2	223–225 dec[c]	55	MeCOMe	$C_{12}H_{15}FN_4O_4S$
3c	F_2CHCH_2	170–173 dec	48	Et_2O	$C_{12}H_{12}F_2N_4$
3d	F_3CCH_2	175–178 dec	51	CH_2Cl_2	$C_{12}H_{11}F_3N_4$

[a] Purity of the compounds was checked by TLC with solvent system B. [b] All compounds were analyzed for C, H, and N. [c] Sulfate salt.

idine polar moieties, present in mifentidine as well as other H_2 antagonists, is discussed.

Scheme I



R	
a	CH_3CH_2
b	FCH_2CH_2
c	F_2CHCH_2
d	F_3CCH_2
e	CN

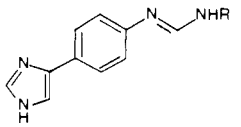
Chemistry

The synthetic pathway followed for the preparation of *N*-(fluoroethyl)-*N'*-(4-1*H*-imidazol-4-ylphenyl)formamidines (**3b–d**) is represented in Scheme I. Imidazolylphenyl isothiocyanate (**1**), obtained by reaction of 4-(5)-(4-aminophenyl)-1*H*-imidazole with carbon disulfide and ethyl chloroformate, was condensed with the requisite 2-fluoro-substituted ethylamines in methanol at room temperature to give the intermediate thioureas (**2b–d**, Table I). Desulfurization of these thioureas by Raney nickel in ethanol furnished the desired formamidines (**3b–d**) in satisfactory yields (Table II).

Results and Discussion

Study Design. The mifentidine molecule possesses a double system of amidine arrangements, one incorporated in the imidazole ring and the other one in a substituted formamidine functionality. Determination of macroscopic pK_a values indicates that this compound may exist in aqueous solution at physiological pH (7.4) mainly in the monoprotonated form at the formamidine moiety. Early studies on derivatives of mifentidine[6] showed that H_2-receptor affinity is enhanced passing from the *N*-methyl-substituted derivative to higher alkyl homologues. The observed gain in potency appears to reflect the increasing hydrophobicity of the substituent. On the contrary, the introduction of a cyano group in place of an alkyl substituent leads to loss of activity ($K_B > 10^{-4}$ M, guinea pig atrium, agonist histamine). The cyano group possesses

Table III. Fragment Constants for Substituents, pK_a Values, and Mole Percentages of Species at pH 7.4 of *N*-Substituted-*N'*-(4-imidazol-4-ylphenyl)formamidines



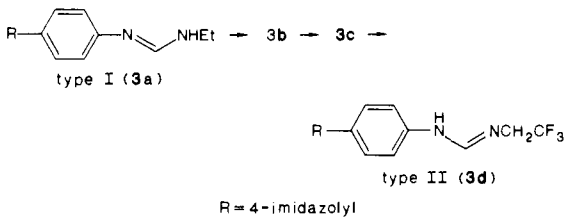
compd	R	f[a]	pK_a		mole % at pH 7.4		
			imidazole	formamidine	dication	monocation	neutral
3a	CH_3CH_2	1.55[b]	5.57	8.65	1.45	93.21	5.32
3b	FCH_2CH_2	0.94[c]	5.55	8.12	1.39	82.60	16.00
3c	F_2CHCH_2	0.93[d]	4.54	6.60	0.13	13.54	86.31
3d	F_3CCH_2	1.31[e]	4.45	6.14	0.11	5.09	94.79
3e	CN	−1.27[f]	nd[g]	<1[g,h]			

[a] Fragment constants of R substituents calculated according to fragment method (see ref 12). [b] $f(CH_3CH_2) = f(CH_3) + f(CH_2)$. [c] $f(FCH_2CH_2) = 2f(CH_2) + f(F)$. [d] $f(F_2CHCH_2) = f(CH_2) + f(CH) + 2f(F) + 2F_{mhG_2}$. [e] $f(F_3CCH_2) = f(CH_2) + f(C) + 3f(F) + 3F_{mhG_3}$. [f] See ref 9. [g] Not determined because of product insolubility. [h] Predicted on the basis of the base-weakening effect of a cyano group attached to α-carbon atoms in amines ($-\Delta pK = 5.8$).

intrinsic hydrophilic character ($\pi = -1.27$);[9] however, its strong electron-withdrawing effect ($F = 0.51$)[9] lowers the basicity of the resulting formamidine ($pK_a < 1$) (Table III). Therefore, it cannot be inferred whether substituent hydrophilicity or decreased basicity of the formamidine is the factor responsible for the detrimental effect on H_2-receptor affinity. Clearly, a better insight into the question would be gained by measuring the activity change brought about by an electron-withdrawing, yet lipophilic, substituent.

The inductive effect associated with a substituent is one of the major intrinsic factors affecting the strength of acids and bases. Since it is known that 2-fluoroalkylamines are significantly weaker bases than their unsubstituted counterparts,[10] and that the fluorine atom has a steric effect comparable to that of hydrogen (van der Waals radius of F = 1.35 Å, H = 1.2 Å),[11] mifentidine derivatives (**3b–d**) containing this type of substitution were considered suitable candidates to assess the importance of formamidine basicity in regard to affinity. In this context, the influence of the fluorine substitution on both lipophilic properties (log P) and pK_a must be considered. Taking the N-ethyl-substituted derivative (**3a**) as a mifentidine model, according to fragment constant analysis,[12] the methyl portion of an ethyl group contributes 0.89 log P ($f(CH_3)$) unit to lipophilicity. Successive substitutions of one and two fluorines in place of hydrogen reduce lipophilicity by 0.61 and 0.62 log P unit, respectively ($f(CH_2F) = 0.28$, $f(CHF_2) = 0.27$). As observed, introduction of a second fluorine affects lipophilicity only slightly, because of dipole shielding following geminal substitution. The shielding effect becomes even greater when a third fluorine is introduced, leading to an increase in the lipophilic contribution ($f(CF_3) = 0.65$). In short, the rank order of lipophilicity for the substituents considered is $CH_2CH_3 > CH_2CF_3 > CH_2CH_2F \geq CH_2CHF_2$, with the trifluoroethyl substituent affording a calculated log P contribution ($f(CH_2CF_3) = 1.31$) not far from that of the ethyl group ($f(CH_2CH_3) = 1.55$) (Table III).

As seen from pK_a values in Table III, successive fluorine substitution affects the basicity of the formamidino group substantially, with a shift in pK_a of 2.5 units going from the N-ethyl-substituted derivative **3a** to the N-trifluoroethyl compound **3d**. Imidazole basicity is decreased to



a lesser extent. Accordingly, species composition at pH 7.4 is remarkably modified throughout the series. It is to be noted that the decrement in pK_a following introduction of fluoroethyl substituents on these phenylformamidines was less pronounced compared to that reported for amine compounds.[10] Conceivably, the decrease in basicity due to transmission of charge by the electronegative fluorine atoms is partly compensated by the progressive change in tautomeric preference from a formamidine of type I to a formamidine of type II intrinsically more basic.[7]

Table IV. H_2 Receptor Antagonist Activity and Mole Percentages of Monocationic Species at pH 6.5 of N-Ethyl- and N-(Fluoroethyl)-N'-(4-imidazol-4-ylphenyl)formamidines

compd	H_2 receptor antagonist activity (guinea pig atria) $K_B \times 10^{-9}$,[a] M (95% CL)	slope (95% CL)	% monocation species at pH = 6.5
3a	177 (82.2–382)	0.99 (0.57–1.40)	88.78
3b	61[b] (40.2–93.3)	0.95 (0.79–1.10)	87.56
3c	21[b] (3.8–119)	0.79 (0.43–1.14)	54.64
3d	7.6[b,c] (3.6–16.1)	0.96 (0.69–1.22)	29.50

[a] 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. [b] Significantly different from **3a**, $P < 0.05$. [c] Significantly different from **3b** and **3c**, $P < 0.05$.

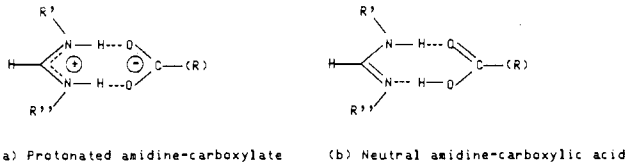


Figure 2. Possible interaction modes of amidine moieties with an acidic site of the H_2 receptor; (R) = receptor; R′ and R″ = amidine substituents.

Biological Results. Affinity of the four probe compounds (**3a–d**) for the histamine H_2 receptor was evaluated from antagonism of the histamine-stimulated chronotropic response on guinea pig atrium. Since the (fluoroethyl)-formamidines were found to decompose in aqueous solution at pH 7.4, the assay was carried out at pH 6.5. At this pH they proved stable for the time required to perform the pharmacological test, as checked by careful HPLC and TLC investigations.

Results showed that fluorine substitution clearly affected receptor affinity. As evident from Table IV, activity was lowest for the N-ethyl-substituted derivative and steadily increased following fluorine substitution. In particular, the trifluoroethyl derivative **3d** proved to be about 20 times as active as the unsubstituted parent compound **3a**.

Discussion. Considering the effect of the above described physical chemical properties on the observed changes in "in vitro" H_2-receptor K_B values (Table IV), the degree of protonation of the formamidino group seemingly plays a role in receptor affinity in the series considered (**3a–d**). The increase in H_2-receptor affinity appears to be inversely related to pK_a values and, hence, to the extent of protonation of the formamidine moiety. As is apparent from inspection of Tables III and IV, the 20-fold difference in affinity between **3a** and **3d**, bearing substituents of similar lipophilic contribution, allows the conclusion that H_2-receptor affinity in this series of phenylformamidines is mostly dependent on the availability of the neutral species. These data lend support to the hypothesis advanced by Haaksma et al.[8] that mifentidine acts through the neutral species.

The model originally suggested by us for mifentidine,[6,7] i.e. binding to an anionic site of the histamine receptor via a charged amidine (Figure 2a), may be reconciled with present findings if one considers the alternative occurrence of a complex involving neutral species (Figure 2b). The driving force for an association of the latter type would

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(10) Henne, A. L.; Stewart, J. J. *J. Am. Chem. Soc.* **1955**, *77*, 1901–2.
(11) Chambers, R. D. *Fluorine in Organic Chemistry*; Wiley-Interscience: New York, 1973; pp 4–25.
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be not the electrostatic charge but rather the decrease in free energy following ring formation through hydrogen-bond pairing. Analogous models have been proposed for associations between nucleic acid bases, which incorporate amidine systems, and carboxylic groups of protein amino acids.[13]

Experimental Section

Chemistry. 2-Fluoroethylamine hydrochloride and 2,2,2-trifluoroethylamine were commercially available (Janssen Chimica); *N*-ethyl-*N'*-(4-1*H*-imidazol-4-ylphenyl)formamidine (**3a**) and *N*-cyano-*N'*-(4-1*H*-imidazol-4-ylphenyl)formamidine (**3e**) were prepared according to a procedure we described previously.[6] Melting points were taken on a Büchi capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian CFT-20 spectrometer operating at 80 MHz in the indicated solvent. Chemical shifts are reported as values relative to tetramethylsilane as internal standard. IR spectra were recorded on a Perkin-Elmer 337 spectrophotometer. TLC were performed on silica gel 60 GF_{254} precoated plates (E. Merck, A.G. Darmstad, Germany) in the following systems: (A) dichloromethane/methanol, 9:1; (B) acetonitrile/acetic acid/water, 8:2:2. All the compounds were analyzed for C, H, N, and S (when present); the analytical results were within ±0.4% of the theoretical values.

2,2-Difluoroethylamine Hydrochloride. A 1 M solution of diborane in THF (82 mL) was dropped into a stirred solution of 2,2-difluoroacetamide[14] (7 g, 73.6 mmol) in anhydrous tetrahydrofuran (70 mL). The temperature was kept at 5–10 °C for 3 h. Ethanol (35 mL) was cautiously added and then gaseous hydrochloric acid was bubbled into the reaction mixture. From the cooled solution, the hydrochloride of the desired compound was obtained as white crystals (4.2 g, 48% yield): mp 248–250 °C dec; NMR (Me_2SO-d_6–$CDCl_3$) δ 8.25 (br m, 3 H), 6.35 (m, 1 H, J = 54 Hz, J = 4 Hz), 3.27 (m, 2 H, J = 15 Hz). Anal. ($C_2H_6ClF_2N$) C, H, N.

4-Imidazol-4-ylphenyl Isothiocyanate (1). Carbon disulfide (9.1 mL, 0.15 mmol) was added to a cooled solution of 4(5)-(4-aminophenyl)-1*H*-imidazole[15] (16 g, 0.1 mol) and 30% ammonium hydroxide (35 mL) in methanol (160 mL) over a period of 15 min. After the mixture was stirred at 0 °C for 24 h, ethyl chloroformate (11.3 g, 0.11 mol) was dropped in at 0 °C. After an additional 15 min of stirring, the mixture was diluted with cold water (200 mL) and the solid which precipitated was filtered and washed with water. This intermediate dithiocarbamate (IR (Nujol) 1750 cm^{-1}; mp 130–132 °C) was quickly converted to the desired isothiocyanate (1) by dissolving it into a solution of Et_3N (6 mL) in methanol (100 mL). The solution was concentrated under vacuum, and water was added to precipitate the title compound as a white solid (7.4 g, 37% yield): mp 198–200 °C; IR (Nujol) 2140–2180 cm^{-1}. Anal. ($C_{10}H_7N_3S$) C, H, N, S.

General Method for the Preparation of *N*-(Fluoroethyl)-*N'*-(4-imidazol-4-ylphenyl)thioureas (Table I, 2b–d). A solution of the proper fluoroethylamine as the hydrochloride (0.15 mol) in methanol (100 mL) was cooled at 5 °C and then a solution of 4% sodium methoxide (0.15 mol) in methanol was added dropwise. After the mixture was stirred for 5 min, 4-imidazol-4-ylphenyl isothiocyanate (1) (0.1 mol) was added portionwise. The reaction mixture was stirred at 5 °C for 3 h and then filtered from the precipitated sodium chloride. The clear solution was evaporated to dryness to give the crude thioureas (**2b–d**), which were purified by crystallization from the indicated solvents (Table I). The NMR data for **2b** were typical: NMR (Me_2SO-d_6–$CDCl_3$) δ 9.55 (s, 1 H), 7.82 (t, 1 H, J = 4.5 Hz), 7.67 (d, 2 H, 8 Hz), 7.67 (s, 1 H), 7.42 (s, 1 H), 7.37 (d, 2 H), 4.53 (m, 2 H, J = 46 Hz), 3.86 (m, 2 H, J = 5 Hz, J = 27.5 Hz).

General Method for the Preparation of *N*-(Fluoroethyl)-*N'*-(4-imidazol-4-ylphenyl)formamidines (Table II, 3b–d). A solution of the requisite thiourea (**2b–d**) (50 mmol) in ethanol (120 mL) was quickly added to a well-stirred suspension of Raney nickel (~50 g) in ethanol (80 mL). The desulfurization reaction was completed in about 10 min, as checked by TLC using solvent B as an eluent mixture. The suspension was then filtered and the clear ethanol solution was evaporated to dryness. The crude formamidines **3b–d** were obtained as white solids and purified by crystallization from the indicated solvents (Table II). The NMR data for **3b**, as sulfate salt, were typical: NMR (D_2O) δ 8.74 (s, 1 H), 8.53 and 8.59 (2 s, 1 H), 7.76 (d, 2 H, J = 8.7 Hz), 7.75 (d, 1 H, J = 1.4 Hz), 7.38 and 7.45 (2 d, 2 H), 4.75 (m, 2 H, J = 56.7 Hz), 3.92 (m, 2 H, J = 1.7 Hz, J = 27.9 Hz).

Determination of Macroscopic Ionization Constants. Macroscopic ionization constants were determined by potentiometric titration in water containing a few milliliters of methanol, according to Albert and Serjeant,[16] by using a PHM Toptronic apparatus, equipped with a DG-111 Mettler electrode. The relative percentage of the ionized formamidines (**3a–d**) at the desired pH were calculated according to the Henderson–Hasselbach equation.[16] The procedure for the determination of the ionization constants of *N*-(2-fluoroethyl)-*N'*-(4-imidazol-4-ylphenyl)formamidine (**3b**) is typical: 152.593 mg of substance (MW = 305.186) accurately weighed, was dissolved in 40.5 mL of previously boiled distilled water and 2 mL of methanol. The initial pH value was recorded and the solution was titrated by adding 0.5 mL of 0.1 N KOH each time corresponding to 0.1 equiv of base (10 mL of 0.1 N KOH = 2 equiv of base). After each addition of KOH, the pH of the equilibrium mixture was recorded. The pK_a values of the other compounds were similarly determined, and the results are given in Table III.

Since following introduction of 2-fluoroethyl groups in the (imidazol-4-ylphenyl)formamidines, the pK_a values of the two basic moieties present in the molecule, i.e. formamidine and imidazole, cannot be unambiguously assigned, *N*-(fluoroethyl)-*N'*-phenylformamidines, lacking the imidazole moiety, were prepared as probe molecules: $C_6H_5N{=}CHNHR$ (**4a**, R = CH_2CH_2F; **4b**, R = CH_2CHF_2; **4c**, R = CH_2CF_3). These compounds were prepared in moderate to good yields by using a procedure similar to the preparation described for **3b–d**, i.e. by reaction of the proper fluoroethylamine with phenyl isothiocyanate followed by desulfurization of the intermediate thioureas (see also Scheme I). Melting points and analytical data for the new compounds were as follows. *N*-(2-Fluoroethyl)-*N'*-phenylformamidine (**4a**): mp 145 °C dec, as picrate salt. Anal. ($C_{15}H_{14}FN_5O_7$) C, H, F, N. *N*-(2,2-Difluoroethyl)-*N'*-phenylformamidine (**4b**): mp 69–71 °C. Anal. ($C_9H_{10}F_2N_2$) C, H, F, N. *N*-(2,2,2-Trifluoroethyl)-*N'*-phenylformamidine (**4c**): mp 80–81 °C. Anal. ($C_9H_9F_3N_2$) C, H, F, N. pK_a values for these probe compounds, determined as described above, were as follows: **4a**, pK_a = 7.8; **4b**, pK_a = 6.9; **4c**, pK_a = 6.3. Comparison of these values with those obtained for **3b–d** allowed the proper pK_a assignment to the formamidino and, hence, to the imidazole group contained in the latter compounds.

Pharmacology. Compounds were administered as their water-soluble salts. Stability of the compounds in the buffer used for the pharmacological assay was checked by TLC using the solvent system B and by HPLC.[17]

Histamine H_2 Antagonist Activity. Atria from guinea pigs (male, Dunkin-Hartley, 400–500 g) were set up in a 50-mL organ bath containing oxygenated (O_2, CO_2 95:5) McEwen's solution (mM: NaCl, 131.6; KCl, 5.6; $CaCl_2$, 2.16; $NaHCO_3$, 24.9; NaH_2PO_4, 1.03; glucose, 11; sucrose, 13) at pH 6.5, 32 °C. Spontaneous beating was monitored with an instantaneous rate meter. Histamine was added to cumulative concentrations until a maximal chronotropic response was observed. Antagonists equilibration time was 60 min. Drug antagonism was evaluated by Schild analysis,[18] fitting linear regressions by least squares and verifying parallelism before dose ratios were calculated. Statistical eval-

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uation of results was performed by the multiple regression method, utilizing "dummy variables" to identify the conditions of validity of the bioassay (parallelism, regression, curvature).

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Registry No. **1**, 56337-96-3; **2b**, 118714-30-0; **2c**, 118714-31-1; **2d**, 118714-32-2; **3a**, 83184-40-1; **3b**, 118714-33-3; **3b**·xH_2SO_4, 118714-36-6; **3c**, 118714-34-4; **3d**, 118714-35-5; **3e**, 83184-32-1; $FCH_2CH_2NH_2$·HCl, 460-08-2; $F_2CHCH_2NH_2$·HCl, 79667-91-7; $F_3CCH_2NH_2$·HCl, 373-88-6; 2,2-difluoroacetamide, 359-38-6; 4-(5)-(4-aminophenyl)-1*H*-imidazole, 29528-28-7; ethyl 4-imidazol-4-ylphenyldithiocarbamate, 118714-29-7; mifentidine, 83184-43-4.

Synthesis and Dopaminergic Activity of 2-Substituted Octahydrobenzo[*f*]quinolines

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A series of 2-substituted octahydrobenzo[*f*]quinolines has been synthesized and assayed for dopamine agonist activity. Only the compounds corresponding to the β-rotameric conformation of dopamine showed biphasic activity in competition binding studies with the radioligand [^{3}H]spiroperidol. These findings suggest that the congeners possessing the β-rotamer conformation show receptor-binding characteristics that resemble those of the ergolines more closely than do those of the corresponding α-rotamer congeners.

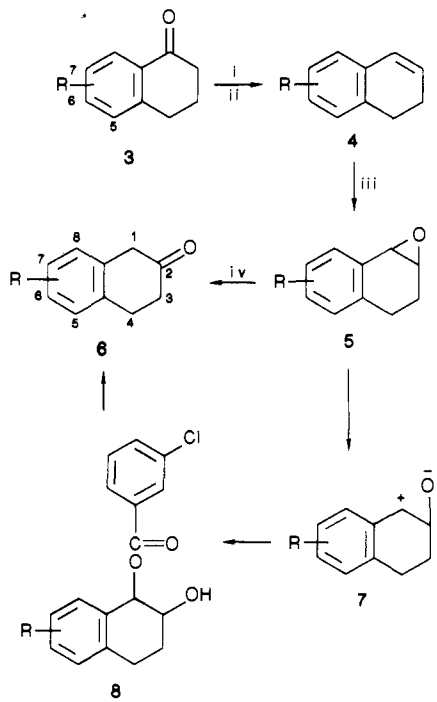
Dopamine agonist drugs have seen a remarkable growth in therapeutic utility during the last few years, following the successful use[1-5] of compounds derived from ergot alkaloids, such as bromocriptine, pergolide (**1**, Chart I), and lisuride, for the treatment of hyperprolactinemia, acromegaly, and Parkinsonism. Increasing efforts have been directed toward the synthesis of new derivatives and partial structures with the aim of isolating the dopaminergic pharmacophore from the multitude of pharmacological effects inherent in the ergo-agonists.[7]

Cannon et al. have reported[6,8] that the octahydrobenzo[*f*]quinolines **2e** were potent dopaminergics. Since the C ring of these compounds, although unsubstituted, is structurally analogous to the D ring of the dihydro ergot alkaloids, it seemed reasonable to propose that the addition of appropriate substituents at the 2-carbon position (corresponding to the 8-position in the ergot alkaloids) would produce more specific dopamine agonists that, while retaining or exceeding the potent dopaminergic activity of the ergot alkaloids, might be free of their undesirable side effects.[9] This was of importance because it is known[10] that the nature of the 8-position in an ergoline profoundly affects its biological properties. For instance, while relatively simple amides of lysergic acid are potent oxytocic drugs, more complex peptide-like amides are vasoconstrictors, the simple diethyl amide (LSD) is a potent hallucinogen, and many variously 8-substituted ergolines exhibit dopaminergic properties.[10] Ten 2-substituted octahydrobenzo[*f*]quinolines related to compounds **2a-d** have been prepared and are reported here to be dopamine receptor agonists. The choice of the 2-substituents was conceptually derived from pergolide ((methylthio)methyl) and lergotrile (cyanomethyl)[6,7] while hydroxymethyl and methyl were chosen for their enhanced and reduced hydrophilicity, respectively.

Chemistry

Preparation of 2-substituted octahydrobenzo[*f*]-quinolines utilized the appropriate 2-tetralones **6** as the starting materials (Scheme I). Reduction and dehydration of 5-methoxy-1-tetralone (**3a**) gave 8-methoxy-1,2-di-

Scheme I[a]



a, R = 5-OMe; b, R = 7-OMe; c, R = 5,6-$(OMe)_2$; d, R = 6,7-$(OMe)_2$

[a] Reagents: (i) $NaBH_4$, (ii) C_6H_6, *p*-toluenesulfonic acid, (iii) *m*-chloroperoxybenzoic acid, (iv) ZnI_2 in C_6H_6.

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